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REPORT AND RECOMMENDATIONS OF THE PANEL TO ASSESS THE NIH INVESTMENT IN RESEARCH ON GENE THERAPY

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Executive Summary of Findings and Recommendations

Dr. Harold Varmus, Director, National Institutes of Health (NIH), appointed an *ad hoc* committee to assess the current status and promise of gene therapy and provide recommendations regarding future NIH-sponsored research in this area. The Panel was asked specifically to comment on how funds and efforts should be distributed among various research areas and what funding mechanisms would be most effective in meeting research goals.

The Panel finds that:

1. Somatic gene therapy is a logical and natural progression in the application of fundamental biomedical science to medicine and offers extraordinary potential, in the long-term, for the management and correction of human disease, including inherited and acquired disorders, cancer, and AIDS. The concept that gene transfer might be used to treat disease is founded on the remarkable advances of the past two decades in recombinant DNA technology. The types of diseases under consideration for gene therapy are diverse; hence, many different treatment strategies are being investigated, each with its own set of scientific and clinical challenges.

2. While the expectations and the promise of gene therapy are great, clinical efficacy has not been definitively demonstrated at this time in any gene therapy protocol, despite anecdotal claims of successful therapy and the initiation of more than 100 Recombinant DNA Advisory Committee (RAC)-approved protocols.

3. Significant problems remain in all basic aspects of gene therapy. Major difficulties at the basic level include shortcomings in all current gene transfer vectors and an inadequate understanding of the biological interaction of these vectors with the host.

4. In the enthusiasm to proceed to clinical trials, basic studies of disease pathophysiology, which are likely to be critical to the eventual success of gene therapy, have not been given adequate attention. Such studies can lead to better definition of the important target cell(s) and to more effective design of the therapeutic approach. They often can be carried out in appropriate animal models. Pathophysiologic studies may also suggest alternative treatment strategies.

5. There is a clear and legitimate need for clinical studies to evaluate various aspects of gene therapy

approaches. Although animal investigations are often valuable, it is not always possible to extrapolate directly from animal experiments to human studies. Indeed, in some cases, such as cystic fibr sis, cancer, and AIDS, animal models do not satisfactorily mimic the major manifestations of the corresponding human disease. Clinical studies represent not only practical implementation of basic discoveries, but also critical experiments which refine and define new questions to be addressed by non-clinical investigation.

6. Interpretation of the results of many gene therapy protocols has been hindered by a very low frequency of gene transfer, reliance on qualitative rather than quantitative assessments of gene transfer and expression, lack of suitable controls, and lack of rigorously defined biochemical or disease endpoints. The impression of the Panel is that only a minority of clinical studies, illustrated by some gene marking experiments, have been designed to yield useful basic information.

7. Overselling of the results of laboratory and clinical studies by investigators and their sponsors--be they academic, federal, or industrial--has led to the mistaken and widespread perception that gene therapy is further developed and more successful than it actually is. Such inaccurate portrayals threaten confidence in the integrity of the field and may ultimately hinder progress toward successful application of gene therapy to human disease.

Based on these findings, the Panel recommends the following:

1. In order to confront the major outstanding obstacles to successful somatic gene therapy, greater focus on basic aspects of gene transfer, and gene expression within the context of gene transfer approaches, is required. Such efforts need to be applied to improving vectors for gene delivery, enhancing and maintaining high level expression of genes transferred to somatic cells, achieving tissue-specific and regulated expression of transferred genes, and directing gene transfer to specific cell types. To stimulate innovative research, the Panel recommends the use of interdisciplinary workshops, specific program announcements in these areas, and the use of short-term, pilot grants for testing new ideas and for encouraging investigators from other areas to enter the field of gene therapy.

2. To address important biological questions and provide a basis for the discovery of alternative treatment modalities, the Panel recommends increased emphasis on research dealing with the mechanisms of disease pathogenesis, further development of animal models of disease, enhanced use of preclinical gene therapy approaches in these models, and greater study of stem cell biology in diverse organ systems.

3. Strict adherence to high standards for excellence in clinical protocols must be demanded of investigators. Gene therapy protocols need to meet the same high standards required for all forms of translational (or clinical) research, whatever the enthusiasm for this (or any other) treatment approach.

4. To enhance the overall level of research in this area, the Panel recommends that NIH support broad interdisciplinary postdoctoral training of M.D. and Ph.D. investigators at the interface of clinical and basic science. Mechanisms for physician training in this area might include use of career development awards based on a program announcement in gene therapy.

5. Investigators in the field and their supporters need to be more restrained in their public discussion of findings, publications, and immediate prospects for the successful implementation of gene therapy approaches. The Panel recommends a concerted effort on the part of scientists, clinicians, science

writers, research advocates, research institutions, industry, and the press to inform the public about not only the extraordinary promise of gene therapy, but also its current limitations.

6. NIH has already provided an appropriate initial investment in gene therapy. Future gene therapy research should compete with other forms of biomedical research for funding under stringent peer review. Only with fair, yet critical, peer review will high standards be met and maintained. The Panel specifically does not recommend special gene therapy study sections, expansion of existing center programs in gene therapy, or expansion of the recently funded core vector production program. To ensure that the level of support remains appropriate, the NIH investment in this field should be reexamined periodically.

7. To enhance the contribution of industry to the field, the Panel recommends that NIH encourage collaborative arrangements between academic institutions and industry that complement NIH-supported research, and also implement mechanisms that facilitate the distribution and testing of vectors and adjunct materials for use in clinical studies.

8. In an effort to improve gene therapy research and reduce duplication of effort, the Panel urges better coordination and scientific review of such research throughout the NIH Intramural Program. In addition, NIH Institute Directors should resist pressures to include gene therapy research in their portfolios (either Intramural or Extramural) to "round out" their programs or compete with other Institutes. Instead, they should include such research only when there are compelling scientific reasons to go forward. Institute Directors should take the lead, where it seems appropriate, to focus efforts on improvement of diagnosis and understanding of disease pathogenesis and await further developments in vector technology before expanding clinical gene therapy programs.

Introduction

Gene therapy is a set of approaches to the treatment of human disease based on the transfer of genetic material (DNA) into an individual. Gene delivery can be achieved either by direct administration of genecontaining viruses or DNA to blood or tissues, or indirectly through the introduction of cells manipulated in the laboratory to harbor foreign DNA. As a sophisticated extension of conventional medical therapy, gene therapy attempts to treat disease in an individual patient by the administration of DNA rather than a drug. Because only somatic cells, and not germ cells (eggs and sperm), are the target of these efforts, gene transfer affects only the individuals under treatment and not their offspring. Therapy directed to germ cells, which would represent a radical departure in the approach to managing disease, is not considered further in this report.

Since genetic material is the putative therapeutic agent, some observers view gene therapy as qualitatively different from other forms of treatment. Seen from a broader perspective, however, somatic gene therapy reflects a natural progression in the application of biomedical science to medicine. In altering the genetic material of somatic cells, gene therapy may correct the underlying specific disease pathophysiology. In some instances, it may offer the potential of a onetime cure for devastating, inherited disorders. In principle, gene therapy should be applicable to many diseases for which current therapeutic approaches are ineffective or where the prospects for effective treatment appear exceedingly low.

Five years have elapsed since the first patients received gene modified cells at the NIH. Since then, the field of gene therapy has attracted increased attention in scientific, medical, media, and lay circles. As of June 1995, 106 clinical protocols involving gene transfer were approved by the NIH Recombinant Advisory Committee (RAC). Indeed, a total of 597 subjects have already undergone gene transfer experiments. Currently, NIH provides approximately $200,000,000 per year for research related to gene therapy. Industrial support of gene therapy research has grown steadily, such that it now is estimated to exceed that of the NIH and underwrites a major proportion of approved clinical protocols. With this high level of current activity the young field of gene therapy is the focus of attention and scrutiny as a frontier of modern medicine.

To advise Dr. Harold Varmus, Director of the NIH, the Panel to Assess the NIH Investment in Research on Gene Therapy (see Appendix A) heard presentations from NIH Institute Directors, basic researchers, and clinical investigators from academic and federal institutions and from the private sector (see Appendix B). The Panel also reviewed recent basic and clinical research in gene therapy.

Panel members are unanimous in recognizing the extraordinary potential, in the longterm, of gene therapy for managing and correcting human disease. Integrating efficacious and workable gene therapy procedures into the health care system would signal a major development in medicine, comparable to past milestones, such as the introduction of aseptic techniques, antibiotics, vaccines, and tissue transplantation. The realization of this long-term goal requires proper development of its scientific underpinnings and validation of its utility to patients with carefully designed, controlled, and evaluated clinical trials.

Although expectations have been great-fueled by the escalating enthusiasm of some investigators, industrial sponsors, and members of the media-it must be recognized that clinical efficacy in human patients has yet to be clearly established for any gene therapy protocol. This sobering reality highlights the challenge of bringing this, or any other, complex technology to clinical practice. Typically, many years are required before new therapies are proved successful. For example, transplantation of bone marrow and other organs--now an accepted therapy for lifethreatening diseases-required more than two decades of development during which frequent failures often provoked widespread skepticism. At this early stage in the development of gene therapy, the Panel considered the following issues:

Is the science underlying the application of gene therapy sufficiently mature to justify rapid and widespread clinical testing? What areas of research need particular development?

Have the clinical trials to date been appropriately designed to be maximally informative? Should stricter standards be adopted?

Is there an appropriate balance between basic and clinical research supported by the NIH?

Are training, research, and resource support mechanisms optimal for nurturing this young field? Should special, targeted research and/or training grant mechanisms be instituted?

What relationships among academic, federal (i.e., NIH), and industrial institutions would best facilitate the development of the clinical applications of gene therapy?

What is the impact of the manner in which investigators in the field and their supporters portray their activities to the scientific community and the public?

In its review the Panel has identified significant problems that need to be addressed. Its recommendations are based on the view that shortcomings must be frankly acknowledged and overcome to realize the full promise of gene therapy.

The rationale for gene therapy of human disease

The concept that gene transfer might be applied to treat disease is founded on the extraordinary advances of the past two decades in the area of recombinant DNA technology. Current methods permit rapid identification and facile manipulation of genes, better enabling investigators to determine the molecular basis of disease and to examine cellular physiology from a molecular perspective. The potential use of gene transfer to treat disease, therefore, is a natural extension of recent fundamental biomedical research.

The types of diseases under consideration for somatic gene therapy are diverse, and have many different underlying causes. Accordingly, the rationales and strategies for treating particular diseases are varied. To assess gene therapy's prospects and status, we, therefore, distinguish among major disease categories.

Single-gene inherited disorders: Many inherited disorders result from mutation of a single gene (hence, singlegene [monogenic] disorders). While individually infrequent in the population, this category as a whole contributes significantly to the chronic disease burden, and includes sickle cell anemia, hemophilias, inherited immune deficiency disorders such as adenosine deaminase deficiency, hypercholesterolemia due to defects in the LDLreceptor, and cystic fibrosis. In many instances singlegene disorders are a direct consequence of loss of function of the relevant protein, such that its replacement (or mere addition to the cell) would be curative. This is the most straightforward application of somatic gene therapy and may be entertained once the mutant gene has been identified and its normal counterpart isolated. Delivery of a normal factor VIII gene to a patient with hemophilia is an example. In some instances, the mutant protein participates more indirectly in cellular pathology, such as in sickle cell anemia where a variant globin causes hemoglobin to polymerize under low oxygen tension, thereby damaging the red blood cell. In this situation, gene transfer and expression of a normal globin chain is still expected to benefit the patient. In yet other instances, such as in dominantly inherited connective tissue disorders in which the presence of an abnormal molecule interferes with normal tissue development and function, only selective silencing of the mutant gene would be expected to be of benefit to the patient.

Although "gene addition" is the simplest strategy for somatic gene therapy, several practical difficulties need to be addressed. Particularly important among these is the need in many instances to deliver the appropriate gene to a specific cell type or tissue. Other challenges includes gaining access to the relevant cell type for correction, assessing the total fraction of cells in a tissue that need to be corrected, achieving the level of expression required for correction, and regulating expression of the added gene once it is transferred into appropriate target cells.

More common, multifactorial disorders: For a variety of more common diseases (e.g., coronary heart disease, diabetes), typically several genes are involved, making a single gene mechanism exceptional. Knowledge of pathophysiology is beginning to suggest how in particular instances the introduction of specific genes might reverse or retard disease processes at the cellular level. This general approach may prove effective regardless of genetic etiology and without the need to replace a single, missing gene product. For instance, in restenosis following angioplasty, local

transfer into vascular cells of genes reducing proliferative and thrombotic processes might prevent reocclusion.

The possibilities for gene transfer as a treatment for common multifactorial diseases are vast. The precise approach needs to be assessed in each instance by considering how specific gene products influence cellular physiology. We can expect many different, sometimes speculative, strategies to be proposed. Each will need to be judged in comparison with conventional treatment approaches.

Cancer: Studies of the past two decades have established cancer as a genetic disease at the cellular level. Cancers arise through a multistage process driven by inherited and relatively frequent somatic mutation of cellular genes, followed by clonal selection of variant cells with increasingly aggressive growth properties. At least three important classes of genes-protooncogenes, tumor suppressor genes, and DNA repair genes-are targeted by mutations. In less than five percent of all individuals with cancer, and a greater percentage of those developing cancer at a younger age, germline mutation of a tumor suppressor or DNA repair gene is a primary determinant for cancer development. However, in contrast to the gene therapy approaches being considered for typical inherited disorders in which a gene product is missing, somatic gene therapy approaches are not suitable for treating those harboring a germline mutation in a cancercausing gene. In these individuals all cells (at least in some tissues) are at risk for cancer development.

The vast majority of mutations that contribute to cancer are somatic, i.e., present only in the neoplastic cells of the patient. The introduction into cancer cells of a gene that might alter or inhibit the malignant phenotype is an appealing concept. It is based, in part, on experimental data showing that introduction of normal copies of tumor suppressor genes (e.g., p53 or Rb) into cancer cell lines *in vitro* restores normal growth properties.

Daunting hurdles must be overcome if gene correction strategies are to achieve a meaningful clinical outcome. First, some cancers arise following mutations in which the gene product has a dominant effect. Hence, transfer of a normal copy of the gene into an affected cell would have little, if any, impact. Second, the number of cells within a clinically detectable cancer is large (>10^9), and the mutation rate within them is so high that mutations in the introduced gene will arise in at least a subset of cells, inactivating its function and resulting in subsequent reoutgrowth of cancer cells. Third, present technologies allow gene transfer to only a subset of cells within a detectable, local tumor mass. Finally, the major dreaded complication of advanced local cancer is distant metastasis, and current means for transferring DNA do not provide feasible strategies for reaching cells that have spread widely in the body.

Because of these formidable problems, other-more indirect-gene therapy approaches to the treatment of cancer are being considered. Included among these are transfer of genes for cytokines or other immunemodulatory products to cancer cells either outside the body (*ex vivo*) or directly into the patient (*in vivo*) in an attempt to stimulate immune recognition of not only the genemodified cancer cells, but also cancer cells that have not received the gene situated elsewhere in the body. In some instances, tumorinfiltrating lymphocytes or other immune effector cells have also been transduced in an attempt to increase their specificity and/or reactivity against tumor cells. Although several of these strategies show promise in mouse models, none has demonstrated efficacy in humans.

A second general approach to the treatment of localized cancers, including brain and liver tumors,

involves *in vivo* delivery to cancer cells of genes encoding viral or bacterial enzymes involved in the conversion of nontoxic prodrugs to their active molecules. In one approach the thymidine kinase gene from herpes simplex virus into cells is transferred into cells, rendering them more susceptible to the drug ganciclovir. Finally, genes that provide enhanced resistance to conventional chemotherapeutic agents are being transferred into bone marrow cells, which are then used to reconstitute the bone marrow of patients before treatment with intensive, and otherwise lethal, chemotherapeutic regimens.

Infectious diseases: In principle, a number of chronic infectious diseases, including several types of hepatitis and herpesvirus infections, may be suitable targets for gene therapy approaches. However, only HIV infection has received much attention to date. Current efforts focus on two general areas: postexposure vaccination in an attempt to boost the host immune response to the infection and attempts to express genes in target cells that render them unable to be infected or of supporting HIV replication. Although a handful of trials are ongoing at present, they are in very early stages, and no results have been published.

In vaccination trials, modified HIV genes are introduced directly into infected individuals following *ex vivo* treatment of target CD4 or precursor cells, typically with retroviral vectors that express genes encoding antiviral products. Several such products are being tested: mutant proteins that inhibit virus replication; antisense RNA that blocks translation of HIV gene products or causes destruction of the HIV genome; ribozymes that attack HIV RNA at specific unique sites; "decoy" RNAs that efficiently compete for binding of viral proteins; and singlechain antibodies that prevent key HIV enzymes from functioning. Although these approaches block HIV replication in cell culture systems, serious obstacles to their practical application remain. Most importantly, it is not yet known what cell types to target, much less how they will be isolated, treated, and returned to the patient. Furthermore, it is unknown whether resistant mutants-the major obstacle to successful drug therapy-will also present a serious problem. Nevertheless, the pursuit of gene therapy remains an active area of acquired immunodeficiency syndrome research, and one that also promises to provide important insights into HIV pathogenesis.

The above discussion illustrates the spectrum of diseases and strategies under consideration for somatic gene therapy and is not meant to be comprehensive. Therapeutic success in most cases will rely on effective gene transfer methods and an understanding of the pathogenesis of each disorder.

Basic science issues in gene therapy

Gene transfer and expression

Somatic gene therapy entails two critical steps: delivery of the gene to appropriate cells and its subsequent maintenance and expression. In this section we review current capabilities for meeting these needs.

Gene transfer: Somatic gene therapy requires the transfer of DNA into recipient cells, either outside the body (*ex vivo*) or by direct administration (*in vivo*). Preferably, this should be accomplished without adverse reactions from the recipient. Ordinarily, the intent is to transfer a gene into host cells where it will reside for a prolonged period. Although in many instances, successful therapy will entail gene transfer to specific cells or tissues, target specificity will not always be required. For example, suitable "generic" cells (such as fibroblasts or myoblasts) may

serve as "manufacturing plants" to produce proteins that function in the circulation (e.g., hemophilia) or are taken up by other body cells (e.g., in some enzyme storage disorders).

Several different systems are in use or under consideration for somatic gene transfer (see Table 1). These include DNA (either naked or complexed), RNA viruses (retroviruses), and DNA viruses (adenovirus, adenoassociated virus [AAV], herpesvirus, and poxvirus). Experience is more extensive with retroviral vectors than with other viruses or nonviral DNA. Each vector system has perceived advantages and disadvantages which influence their selection for current or projected clinical applications (see Table 1). Unfortunately, none of the available vector systems is entirely satisfactory, and many of the perceived advantages of vector systems have not been experimentally validated. Until progress is made in these areas, slow and erratic success in applying gene transfer methods to patients can be expected.

The basic biology of retroviruses is the best understood of the vector systems used for gene transfer experiments. Accordingly, retroviruses are employed in the majority of clinical protocols (see Table 2). Among their advantages are efficient entry into dividing cells and integration of the transferred genetic material into the host genome without concomitant introduction of viral genes. Retroviruses would appear to be most suitable for permanent correction of genetic diseases. A major disadvantage of retroviruses is that they infect and integrate only dividing cells. Other problems include cumbersome preparation and relatively low titer, size constraints on inserted genes, difficulties in controlling or ensuring expression, and the potential for genetic damage due to random integration in the host genome.

The adenovirus vector system has found advocates more recently. Among its advantages are high titers and levels of expression, relative ease of handling, efficient infection of many types of human cells, and capacity to infect nondividing cells. Major disadvantages include its relatively high immunogenicity and the complexity of its genome. Despite the widespread belief that adenovirus does not integrate into the host genome, experimental evidence for this assertion is lacking. The persistence and expression of adenoviruses *in vivo* in somatic gene therapy situations are under investigation in several laboratories.

Experience with other DNA viral systems is less extensive. A major perceived strength of AAV is integration at a specific site in the infected cell genome, a finding confirmed thus far only for the wildtype virus. Research with AAV and herpesvirus has been impeded by the lack of suitable helper cell lines for preparing large amounts of pure, recombinant virus. Poxviruses appear most suitable for vaccination.

Direct administration of DNA or DNA complexes (e.g., liposomes) *in vivo* is in its infancy. The ease of preparation and virtually unlimited size of constructs for gene delivery make this approach attractive. The lower efficiency of gene transfer (compared with viruses) and the absence of mechanisms for specifically maintaining the introduced DNA within the cell are major disadvantages. However, the use of naked DNA for *in vivo* vaccination appears feasible and highly promising.

Rather than delivering a particular gene to all cells *ex vivo* or to a specific tissue *in vivo*, it appears preferable to target gene transfer to a particular cell type. In principle, this might be accomplished by incorporating ligands for cell surface receptors into viral envelopes or DNA complexes. However, such strategies have not yet reached clinical application.

Of the vector systems studied to date, retroviruses appear to be most suited for delivering genes to host cells in a stable form due to the efficient integration of retrovirally transduced genes. Studies of yeast cells have defined many of the components necessary for maintaining chromosomes within cells. In principle, the development of artificial human chromosomes as vectors might allow for maintenance of transferred genes without the problems resulting from random insertion of foreign sequences into the host genome. Several laboratories are trying to design such vectors. The efficient introduction of these vectors into cells, however, is likely to be a formidable obstacle to their use for gene therapy in the foreseeable future.

Expression of transferred genes: Expression of transferred genes is essential for successful gene therapy. Much is known regarding DNA sequences that direct highlevel, tissuespecific expression of genes in cells in tissue culture or in transgenic mice. In practice, highlevel expression of genes transferred to somatic cells may not persist or be consistently achieved. Whether these difficulties reflect undefined cellular mechanisms that repress virally introduced genes, a subtle selective disadvantage of stem cells expressing transferred genes, or the failure to include appropriate positive regulatory sequences in the constructs is unknown.

These uncertainties point to the relative dearth of wellcontrolled studies of appropriate and sustained gene expression following somatic gene transfer into animals. In many of the published reports in this field, gene expression was monitored by highly sensitive surrogate methods (e.g., cellular resistance to the drug G418 or reverse-transcriptase PCR assay), rather than by direct measurement of the desired protein product by immunologic or enzymatic activity. This practice reflects the generally low absolute level of gene expression achieved in many instances, leading to a reliance on nonquantitative analyses.

How have some of these problems of gene transfer and expression been reflected in gene therapy experiments involving animals and human subjects? Studies of retrovirusbased gene transfer into hematopoietic stem cells provide one perspective. In mice, current protocols permit transfer of genes into a substantial fraction of stem cells following retroviral infection of marrow cells *ex vivo*. Nevertheless, gene transfer into marrow stem cells of other species (including humans, other primates, and canines) has been much less efficient, with 10% or fewer cells transduced. In clinical protocols to date, the low efficiency of gene transfer is particularly notable. This inefficiency reduces potential benefits of introducing a particular foreign gene, and interferes with efforts to measure expression *in vivo*. Hence, both the clinical benefit and scientific value of clinical trials are compromised.

Current data are largely inadequate with respect to experimental study of the expression of transferred genes. In mouse experiments, longterm expression of transferred genes has been reported, but the consistency of achieving such results is unknown. Also, the quantitation of levels of gene expression over time has not received adequate attention. In human trials, the extent of gene expression is uncertain. In many instances, the efficiency of gene transfer is so poor that investigators have relied on highly sensitive molecular methods (such as reverse transcriptase PCR) rather than biologically more meaningful protein assays, to evaluate expression *in vivo*.

Appropriate tissue expression and recipient cells: Gene therapy approaches would be appreciably enhanced by directing gene transfer and/or expression to the appropriate cells of the body. *Ex vivo* approaches help to ensure that gene transfer is limited to cells of a particular organ. For example, gene transfer into bone marrow cells provides a means to introduce genes selectively

into various blood cell types, including hematopoietic stem cells. Providing a gene product to distinct cell types *in vivo* necessitates either targeting of gene transfer to specific cells or selective expression of introduced genes in specific cell types. To approach the former problem, research aims to incorporate ligands for cellular receptors into viral envelopes or achieve cellspecific gene transfer by binding of virus and target cells to particular proteins or fusion proteins. Meanwhile, tissuespecific gene expression of transferred genes may be accomplished by including appropriate regulatory sequences in gene transfer vectors. Some of these regulatory sequences may be responsive to drugs; hence, *in vivo* expression of transferred genes might be regulated by administration of the relevant drug to the host. Research in these areas within the context of gene therapy strategies is in its infancy.

Disease pathophysiology

Cloning genes and characterizing mutations responsible for human disorders are but two of the essential steps in understanding disease pathogenesis. Defining the mechanisms by which mutations lead to pathology is important in conceptualizing approaches to therapy. For example, some mutations may abolish gene function; in these situations, replacing the missing protein may provide adequate therapy. Alternatively, mutations may alter protein function so as to inhibit a cellular pathway (a dominantnegative mechanism). In these instances, shuttingoff expression of the mutant protein or interfering with its function might constitute therapy.

A basic understanding of the pathophysiology of disease is therefore highly relevant when designing gene therapy strategies. Besides understanding how a mutation leads to disease, it is important to determine which cells of the body are suitable targets for effective therapy. Disorders resulting from the deficiency of a circulating protein (e.g., clotting factors VIII or IX in hemophilia) might be corrected by expression of the relevant gene in skin or muscle cells, even if the protein is normally made in liver, as long as it is secreted into the bloodstream. In many other situations, expression of a transferred gene is required in a particular tissue. For example, correction of primary hemoglobinopathies, such as sickle cell anemia and Cooley's anemia, necessitates precisely regulated expression of globin chains in developing red blood cell precursors. For cystic fibrosis, which is due to loss or malfunction of a membrane protein (CFTR), it is relevant to ascertain which, and how many, cells of the lung need to express a normal CFTR gene.

Study of disease pathogenesis may sometimes lead to the development of highly effective new therapies, as illustrated by now classic research on the biochemical basis of hypercholesterolemia. Elucidation of feedback regulation of cholesterol biosynthesis led directly to the testing of HMGCoA reductase inhibitors as cholesterol lowering drugs. These agents, which are in use worldwide, have been shown to be effective in preventing cardiovascular disease. In the current climate, where the cloning of a new disease gene is often viewed principally in the context of gene therapy, the discovery of these drugs might not have been made.

Animal models of disease

Principles of disease pathogenesis and the development of gene therapy approaches can often be addressed by studying animal models of human disease. Specific hypotheses and experimental therapies should generally be tested extensively in small animals prior to human experiments. The following questions are representative of those that may be profitably addressed in animal experiments. Can particular cell types serve as appropriate targets for gene therapy? Can bone marrow expression of a

gene product whose deficiency leads to a storage disorder affecting the brain improve central nervous system function? What fraction of cells of a tissue need to be altered genetically in order to effect clinical improvement? Are gene modified cells at a selective advantage or disadvantage *in vivo*? Does the host develop an immune response to the gene transfer vehicles or to the newly introduced gene product? Animal models can provide an important link in the development of gene therapy approaches, lying between gene discovery and characterization and clinical experiments. Animal models also constitute a valuable resource for testing other forms of therapy that are not based on gene transfer approaches.

Animal models for genetic diseases have arisen spontaneously in a variety of species (e.g., mouse, cat, dog). Using new methods to mutate genes in embryonic stem cells, mice with engineered alterations in any given gene can be produced. Numerous mouse strains with mutations in genes relevant to human diseases have already been created in this manner, and also by injection of human genes into fertilized mouse eggs. In some instances, mice with such mutations exhibit a phenotype similar to that seen in humans (examples: chronic granulomatous disease, hemophilia A, spinocerebellar ataxia1). In others, the effects of specific mutations in the mouse appear more severe than in humans (examples: ADA deficiency, Gaucher's disease).

Unfortunately, however, mouse models often do not faithfully mimic the relevant human conditions. For example, hypoxanthine phosphoribosyltransferase deficiency associated with LeschNyhan disease in humans is benign in mice due to the presence of an alternative metabolic pathway. Mice with mutations in the CFTR gene do not exhibit the pulmonary effects of cystic fibrosis seen in man, but rather suffer from severe gastrointestinal obstruction. Studying the differences between human diseases and animal model phenotypes may provide insights into disease pathogenesis that may, in turn, be exploited either by gene therapy or pharmacological approaches. Animal models for many cancers and for HIV infection have also been developed. In these instances, the relevance of animal models to human disease appears less certain than in typical singlegene disorders.

Despite potential phenotypic differences between human patients and animal models of disease, the study of animal models for the design of gene therapy approaches in a preclinical setting is important and should not be undervalued. As additional genes leading to human diseases are isolated, and gene targeting and transgenic technologies generate more mouse models of various human diseases, we should anticipate an increasingly productive use of such models to elucidate disease pathophysiology, possibly leading to gene therapy approaches.

Confidence in current approaches to somatic gene therapy would rise if a genuine genetic deficiency in an animal were unequivocally corrected. Although genetic defects in animals have been corrected by introducing transgenes into the germline (or by interbreeding with transgenic animals), somatic gene transfer has not permanently corrected a genetic disease in an animal (e.g., a mouse model of a singlegene disorder).

Recommendations for Basic Science Research:

1. Given the central role of vectors for delivering genes to somatic cells for therapeutic purposes, the Panel endorses *vigorous* and *expanded* research aimed at developing improved vectors. Special emphasis should be placed on the development of viral and nonviral vectors suitable for gene therapy approaches, stable nonintegrating vectors (e.g., artificial chromosomes), vectors capable of efficient gene transfer into nondividing cells, and vectors designed for tissuerestricted targeting and/or regulated

expression.

It is unlikely that a single vector will prove optimal for all gene therapy approaches. We, therefore, urge the NIH to support wideranging research in vector development and allied areas. An understanding of the behavior of vectors and the fate of DNA introduced into somatic cells will require basic efforts in virology, cell biology, immunology, and the chemistry of DNA complexes. These efforts should also include novel approaches to the selective inhibition of gene function including, but not limited to, the continued development of antisense and ribozyme strategies.

2. To facilitate interdisciplinary efforts to develop optimal vectors, the NIH should consider several strategies, including workshops and program announcements, to stimulate discovery, interchange, and collaboration among scientists in diverse areas.

3. The Panel finds that very little research effort is focused on understanding the mechanisms that govern maintenance or shutoff of gene expression following gene delivery in gene therapy experiments. Available data are largely anecdotal. We urge the NIH to give high priority to basic research to elucidate how recipient cells, and particularly stem cells, handle and express foreign DNA sequences.

4. The Panel urges expanded NIH research into the biology of stem cells in diverse organ systems, as such cells are particularly favorable recipients for permanent correction of monogenic disorders. Specific topics include identifying and enriching stem cells from various organs, targeted transfer into and expression of genes in stem cells, the discovery of growth factors required by stem cells, and methods for selectively modifying genes in stem cells.

5. In the enthusiasm to begin human gene therapy trials soon after gene discovery, important aspects of disease pathophysiology, cell biology, and biochemistry have often been underemphasized. Better elucidation of these aspects will reveal the nature of the target cells within a tissue that need to receive the transferred gene, potential difficulties in achieving gene transfer into the appropriate cells or tissue, and features of the relevant protein that may be critical for its function *in vivo*. This increased focus on basic mechanisms of pathophysiology should also foster alternative efforts to develop pharmacological approaches to disease management. We recommend that the NIH vigorously support basic research into molecular mechanisms that produce disease. The present enthusiasm for molecular approaches to therapy, no matter how justified, must not lead to neglect of biochemical and pathophysiologic mechanisms at the tissue and organ level, which may lead to novel therapeutic insights.

6. We recommend that NIH provide continued and expanded support for the development and study of those animal models of disease that faithfully reflect the corresponding human disorders. These models should strengthen the preclinical scientific basis for gene therapy protocols. This approach will often be more costeffective than attempting to perform similar studies in humans.

Gene therapy in man Status of the field

More than 100 clinical protocols for gene therapy have been reviewed and approved by the RAC and subsequently approved by the NIH Director (Table 3). Indeed, 597 individuals have already undergone gene transfer in experiments involving more than a dozen diseases. The majority of human gene transfer protocols involve some form of cancer, rather than the treatment of inherited disease. A proportion is designed as "gene marking studies" that utilize cells "marked" with an introduced gene to track the cellular origin of tumor recurrence. Retroviruses are employed as gene transfer vehicles in the majority

of protocols (Table 2).

Although widely referred to as "clinical trials," gene transfer protocols to date are in truth smallscale clinical experiments. Such exploratory studies are meant to test the feasibility and safety of administering particular vectors and to evaluate the effects of expressing specific gene products. Because these studies have not been designed to measure efficacy, they do not include sufficient controls to evaluate the true merits of gene therapy or compare this approach with conventional approaches to the same disease.

Only a few of these clinical studies are designed well enough to address fundamental biological questions. Most notable are several elegant gene marking studies investigating the cellular origin of tumor recurrence and other recent studies comparing the relative survival of cells of HIV-patients simultaneously infected with different retroviruses meant to inhibit HIV replication. These well designed studies greatly increase the information that may be extracted from careful clinical experiments involving only a few patients.

Upon reviewing the status of clinical protocols approved for gene transfer the Panel made several observations:

Efficacy has not been established for any gene therapy protocol. For example, the administration of PEGADA (a preparation of the enzyme adenosine deaminase that is stable *in vivo*) to patients with adenosine deaminase deficiency, though clinically appropriate in light of its demonstrated efficacy, complicates evaluation of patients initially treated with retrovirally transduced lymphocytes and infants more recently treated with transduced cord blood cells. Furthermore, the atypical, rather mild clinical symptoms of some of the first patients before the experimental procedure began complicates any assessment of its effects. In the case of gene transfer for another disorder, treatment results in five patients with homozygous familial hypercholesterolemia were inconsistent and disappointing with only slight or no changes in cholesterol metabolism and levels.

Adverse short term effects related to gene transfer protocols appear to vary, depending on the nature of the virus used as a vector and the patient to which it is administered. For example, the use of retroviruses in patients with adenosine deaminase deficiency and in marker studies has not been associated with any obvious adverse effects. However, administration of high titer adenovirus vectors to patients with cystic fibrosis has been associated with severe host inflammatory responses.

- Because clinical experience is still so limited, it is not possible to exclude longterm adverse effects of gene transfer therapy, such as might arise from mutations when viral sequences randomly integrate at critical sites in the genome of somatic cells. It must be noted that multiple integration events resulting from repeated administration of large doses of retroviruses theoretically pose a risk for leukemic transformation. Only longitudinal clinical followup of treated patients can provide data on the long term safety of gene therapy protocols.

  Assessment of the results of gene therapy protocols has been hindered in the majority of studies by the low frequency of gene delivery to target cells and the lack of definable biochemical or clinical endpoints.

  Expectations of current gene therapy protocols have been oversold. Overzealous representation of

clinical gene therapy has obscured the exploratory nature of the initial studies, colored the manner in which findings are portrayed to the scientific press and public, and led to the widely held, but mistaken, perception that clinical gene therapy is already highly successful. Such misrepresentation threatens confidence in the field and will inevitably lead to disappointment in both medical and lay communities.

Of even greater concern is the possibility that patients, their families, and health providers may make unwise decisions regarding treatment alternatives, holding out for cures that they mistakenly believe are "just around the corner." For instance, patients with cystic fibrosis may be less vigilant about pulmonary management or a couple at risk for producing a child with a lifethreatening genetic disorder may base reproductive decisions on unrealistic expectations of gene therapy. These reallife scenarios illustrate how patients and their families are placed at risk if the information provided to them is overly optimistic regarding the actual development of successful gene therapy.

In view of these and other difficulties, the Panel considered the appropriateness of clinical studies of gene therapy at this time. The consensus view of the Panel is that clinical studies are warranted for several important reasons-precisely those that distinguish basic and clinical investigation:

It is not always possible to extrapolate results from experiments in animals to human studies. This difficulty is particularly evident with respect to the efficiency of gene delivery and the host response to viral vectors. Although primate experiments might substitute for some human studies, they entail extraordinary costs for meeting animal care needs, and are not entirely adequate for addressing many key issues.

Animal models are not satisfactory for studying many important human disorders, including cystic fibrosis, various cancers, and AIDS. Therefore, human studies are necessary to develop effective treatments for these and many other diseases.

Clinical gene therapy studies reveal problems and raise questions that cannot be otherwise anticipated. For example, in the cystic fibrosis studies the magnitude of the host response to adenoviral vectors was underestimated. This realization has directed research efforts toward engineering vectors that cannot express viral gene products and modulating host responses pharmacologically. Such research may have a substantial impact on gene therapy approaches to other diseases.

- Gene therapy clinical research may provide insights into fundamental disease pathology that may direct subsequent treatment approaches. For example, results from gene marking studies permit investigators to design strategies for purging residual cancer cells from the bone marrow of patients. Reciprocal and synergistic relationships between clinical studies and basic research may emerge from initial clinical gene transfer studies.

Many of the issues faced in bringing gene therapy to clinical practice are encountered when any recent discoveries are applied to the management of disease. The success of such endeavors (often termed "translational research") relies on the quality of the underlying science, the care with which clinical protocols are designed, the melding of different disciplines and strategies into a cohesive approach, and the capacity of investigators to bridge science and medicine. Research at the interface of frontier science and patient care is challenging, and requires that investigators have broad training and biological perspective. For this and other fields of clinical investigation to succeed, high standards of experimental design and robust methods for evaluating clinical outcomes are needed. In the Panel's judgment, many

clinical gene therapy studies thus far have not met these standards.

Recommendati ns:

1. The Panel insists on the adherence to rigorous standards for what constitutes appropriate and meaningful human experiments or clinical trials. Inadequacies of many clinical studies to date result from insufficient attention to research design, poorly defined molecular and clinical endpoints, and lack of rigor. All studies should define molecular, biochemical, and quantitative clinical endpoints. They also need to address specific hypotheses, enabling investigators to interpret negative as well as positive findings. These standards are no different from those required for other forms of translational clinical research. Relaxed standards are unacceptable and cannot be excused by unbridled enthusiasm for this treatment modality.

2. The Panel endorses efforts to develop broad, interdisciplinary training programs in clinical (or translational) research (see below). Training of clinical investigators with broad experiences in biomedical and clinical activities, including biostatistics, will benefit not only the immediate field of gene therapy, but also other areas of translational research.

3. The Panel urges gene therapy investigators and their sponsors--be they academic, governmental, private, or industrial-to be more circumspect regarding the aims and accomplishments of clinical protocols when discussing their work with the scientific community, the public, and the media.

Research training and public education

The development of successful gene therapy approaches necessitates involvement of multiple research and clinical disciplines. Few basic scientists are broadly educated regarding the clinical challenges. Similarly, many clinical scientists, and particularly practicing clinicians, are not sufficiently informed regarding the scientific problems faced in gene therapy. As the field of gene therapy expands, the need for appropriately trained professional personnel, including basic scientists with familiarity of disease pathophysiology and medical scientists and physicians with an appreciation of the complex basic science issues, will become even greater.

We cannot predict when the clinical benefits of gene therapy will be realized. The Panel senses that the public has little understanding of the enormous challenges in the field, and may believe its day has already come, or is at least imminent. Raising such false hopes threatens public support, particularly if effective therapies for more common disorders are not quickly delivered, and may encourage patients and their families to make unwise decisions regarding their treatment options. Scientists, clinicians, scientific journalists, and the press need to devote more attention to responsible, public education regarding the current status and prospects for gene therapy.

Recommendations:

1. The challenging issues faced in clinical applications of gene therapy are common to different areas of medicine. The Panel recommends vigorous support of programs at the postdoctoral level that will combine rigorous training at the interface between clinical and basic science. These programs, which are envisioned to include both M.D. and Ph.D.trained individuals, should not be restricted to the field of gene therapy, but should encompass translational research of all kinds. Mechanisms for physician training in this area could include the use of career development awards based on a program

announcement.

2. The Panel recommends a concerted effort on the part of scientists, clinicians, science writers, research advocates, research institutions, and the press to inform the public regarding not only the great promise of gene therapy but also current realities. This program of education needs to stress that some time will be required to develop the science of the field and to translate these advances to clinical practice.

3. The Panel urges those who care for patients and provide advice regarding treatment and reproductive options to present the current capabilities of the gene therapy field in an honest and restrained manner. Otherwise, patients and their families may fail to utilize more conventional therapies from which they may receive substantial clinical benefit or choose reproductive options based on unrealistic expectations of curative gene therapy.

Resources

Gene therapy depends on multiple resources for generation of approved vectors for clinical use and for clinical management of treated patients. A perceived impediment to the initiation of clinical protocols is the high expense of producing viral vectors that meet good manufacturing practice (GMP)standards. Production of retrovirus for clinical use costs $100,000 or more, an amount beyond the budget of most laboratories or academic institutions. In most instances, vectors have been prepared on contract, often by industry. In response to requests from the gene therapy community for resources for vector production, the NIH funded three central vector production facilities. These sites represent a modest NIH investment in this area that cannot realistically fulfill all requests for vector production. Uncertainties regarding which vectors may be best suited for specific clinical studies argue against establishing a large national infrastructure for vector production. Instead, the use of the recently funded program should be critically evaluated and assessed periodically. Meanwhile, the vector production sites should pay particular attention to applications requesting vectors for use in protocols that emphasize rigorous experimental design and the testing of hypotheses, rather than those that duplicate efforts of other institutions. Furthermore, the relative costs of vector production at the NIHsupported sites should be carefully compared with those incurred in producing vectors under contracts to industry. Only then will it be possible to determine the value of the NIH's investment in vector production.

Resources currently exist at many institutions for the performance of clinical studies related to gene therapy. The NIH-supported general clinical research centers (GCRCs) represent a highly appropriate resource for the community.

Recommendations:

1. The Panel does not endorse the expansion of the NIHsupported vector core program at this time. The need for additional resources in this area should be reexamined periodically.

2. For clinical studies, the Panel urges that investigators make efficient use of NIHsupported GCRCs. These centers have been established to promote research at the interface of clinical and laboratory sciences and are well suited for use in human clinical trials.

Grants and review process

If gene therapy is to develop as a practical and useful treatment option, major improvements in diverse

areas-including vector systems, gene expression following gene transfer, identification and manipulation of stem cells, generation of appropriate animal models of human diseases, and study of disease pathogenesis-are needed. The Panel discussed the relative merits of different strategies for promoting research excellence. Would needs be best served by establishing additional centers for gene therapy, perhaps organized around specific diseases or organ systems? Or should gene therapy research proposals compete more directly with other forms of research for funding? To what extent do grants in the field of gene therapy receive a fair review, especially given the multidisciplinary nature of the studies? In the Panel's judgment, the best way to foster high quality research and innovation is through competitive peer review, rather than by reliance on special support mechanisms.

NIH has already provided the field of gene therapy with an appropriate start by support of gene therapy centers and specific requests for applications (RFAs). The Panel believes that the current level of research support for this area of biomedicine is appropriate at this time, and suggests that funds for future efforts be allocated on the basis of traditional peer review to ensure that current problems in the field are addressed critically. The adequacy of funding for clinical protocols, particularly outside the NIH campus, has been difficult to assess, since a substantial proportion of support is currently provided by industry. We see no indication that clinical applications in the field of gene therapy are being held back by inadequate financial support.

Recommendations:

1. The Panel endorses efforts to ensure that rigorous peer review of gene therapy is imposed at all levels, from basic research to clinical trials.

2. To guarantee sound review of gene therapy proposals, particularly those which include clinical studies, the Panel urges that membership of NIH study sections be broadened so that they are better able to review both basic and applied aspects of projects. This view is in agreement with the recommendations of the committee chaired by Keith Yamamoto that recently evaluated the peer review system at the NIH.

3. The Panel recommends that gene therapy research compete directly with all other forms of therapeutic research for funding. Because different approaches may lead to successful treatment of disease, it would be unwise to focus only on one approach, such as gene therapy, for special support.

4. The Panel opposes the formation of study sections dedicated to the review of proposals in the area of gene therapy. If high standards are to be met, research in this area needs to compete with that in other fields of biomedical science.

5. The field of gene therapy should be reviewed periodically to assess whether the investment by NIH should be increased or decreased.

6. To stimulate truly innovative research, the Panel recommends that several Institutes of NIH pool funds through the R21 grant program for short pilot projects focused in specific areas, including vector design and expression of transduced genes, animal models of disease, and stem cell biology.

7. Although it did not formally evaluate the role of RAC, in evaluation of clinical protocols, the Panel recognizes the need for continued review of the safety of gene therapy by expert scientists.

Role of industry in gene therapy research and clinical activities

Industry substantially influences gene therapy. The field includes both small biotechnology firms which have emerged as a result of activities of academic or NIH investigators, and larger biotechnology companies and traditional pharmaceutical corporations. *In toto*, the research support provided by industry exceeds that of NIH. Therefore, industry plays a major role in the area of gene therapy, one that is certain to increase in future years.

Industry has important attributes that recommend its active participation in gene therapy. First, industry is skilled in translational research and the development of drug products. Second, it has significant experience in meeting high manufacturing and quality control standards, and maintains a professional staff dedicated to regulatory and clinical issues. Third, a high level of scientific and technical expertise characterizes modern biotechnology and pharmaceutical companies.

Several companies have ongoing research programs developing improved vectors for gene delivery and better systems for expression of foreign genes. Moreover, industry has been the major supporter of many of the approved clinical protocols. It is axiomatic that success for biotechnology or pharmaceutical companies will be equated with the development of FDAapproved, clinically efficacious gene transfer treatments for disease. Industrial efforts will focus where the perceived use of the product is greatest, and likely to yield high profits. Hence, industry will tend to concentrate on common diseases, such as cancer, rather than rare disorders. This imbalance has not been evident thus far, as some companies are studying rare diseases initially, aiming to demonstrate proof of concept. For example, industry is supporting clinical studies of adenosine deaminase deficiency, Fanconi's anemia, and cystic fibrosis. Once clinical efficacy of gene therapy procedures is demonstrated for specific, infrequent disorders, however, it can be anticipated that market forces will drive industry's involvement toward common diseases for which patient populations are large.

Industry is collaborating with academic institutions across a wide spectrum. On the whole, this involvement is healthy and complements NIHsupported research. For example, industrial partners have prepared GMPgrade vectors for many clinical studies at academic institutions. The development of gene therapy as a clinical activity is threatened, however, by potential conflicts among the demands of good science and the goals of academic researchers, clinicians, industry, and its investors. The field is at risk to the extent that the premature initiation of clinical studies and overzealous, uncritical reports of clinical results are used by industry to promote investment and perceived research dominance. Likewise, if the objectivity and integrity of academic investigators associated with specific companies is undermined as they seek to maintain their industrial ties, the field will be jeopardized. Decisions regarding diseases to be treated need to be made by investigators on scientific rather than financial criteria. Although the problems of conflict of interest in the field of gene therapy do not differ substantially from those encountered in other forms of clinical research, the wide publicity given to clinical gene therapy efforts raises the potential stakes for both academic investigators and those at companies.

For the future development of the field it will be important that issues of proprietary control not limit the development of clinical protocols. The Panel heard several presentations that described logistical difficulties encountered in gaining industrial approval to perform clinical studies in which cytokines and other reagents were to be obtained from several, often competing companies. These obstacles would be reduced if mechanisms were developed to facilitate the dissemination of useful materials for clinical trials.

In the opinion of the Panel, it is premature to assess what impact, if any, the licensing of a broad patent to a single company for *ex vivo* gene therapy will have on the field. The Panel is concerned, however, that broad patents of this kind will ultimately retard implementation of successful gene therapy protocols once they are developed. Additional study of the impact of patents on the development of the field will be necessary.

Recommendations:

1. The Panel urges the NIH to maintain support for peerreviewed research in gene therapy and clinical trials, particularly in areas that may not receive attention from the industrial sector, such as the development of gene therapy for rare inherited disorders.

2. NIH should encourage collaborative arrangements that complement NIHsupported research. Industry can play an important role in providing GMPgrade vectors for clinical testing and in designing clinical trials that meet rigorous criteria for efficacy and regulatory standards.

3. The Panel urges the NIH to develop and implement mechanisms that would facilitate the distribution and testing of adjunct materials (e.g., cytokines) for use in gene therapy.

Intramural NIH support of gene therapy

The first human gene transfer experiments were performed at the NIH, and have engendered excitement within the Intramural Program. At present, more than 100 Intramural investigators are engaged in research pertaining to gene therapy. A much higher proportion of the NIH Intramural research budget than the Extramural budget is devoted to gene therapy, according to information provided by the Institute Directors (5% vs. 1% overall). New collaborative arrangements within the Intramural Program are emerging. Clinical protocols addressing several different disorders, including Gaucher's disease, chronic granulomatous disease, Fanconi's anemia, and cancer, have been approved.

In their presentations to the Panel, Institute Directors discussed and spoke highly of research programs in gene therapy. Of these presentations two aspects are noteworthy. First, there appears to be little coordination of research across Institute boundaries, such that duplicative efforts are inevitable. Second, much of the research utilized similar, yet inadequate, vector systems, which were tailored to deliver genes to the tissue of each Institute's interest. In these respects, Intramural research does not differ from that taking place elsewhere. Research of this kind, however, is unlikely to provide innovative advances. Institute Directors should be encouraged to support innovative research approaches, whether they be Intramural or Extramural, in whatever field of endeavor, even if this leads to deemphasis of gene therapy research within an Institute. They should resist the temptation to fill the "portfolio" with research that appears "hot" but may lack a strong scientific basis or likelihood of success relative to other areas.

The Clinical Center of the NIH campus is a superb resource for the execution of clinical investigation at all levels. With a new clinical center, currently under development, the NIH would be assured firstrate facilities well into the next century. The NIH Clinical Center and its staff have proved effective over the years in attracting and maintaining a patient base representing a wide spectrum of diseases, including many rare, inherited disorders. As such, it is an excellent resource, both for the Intramural Program and the country. The recent decline in patient occupancy in the Clinical Center is a cause for concern, which is being appropriately addressed. It is hoped that erosion of the excellent patient resource base of the NIH will not occur, so that clinical investigation in the Intramural branch will not be jeopardized.

Recommendations:

1. The Panel appreciates that the concentration of talented basic and clinical investigators on the NIH campus provides an extraordinary resource for gene therapy research. However, better coordination and scientific review of gene therapy research throughout the NIH campus is needed. Improved coordination and review will foster research excellence and reduce duplication of effort at a time of budgetary constraints.

2. The Panel urges Institute Directors to include gene therapy within their portfolios only when there are compelling scientific reasons. Accordingly, they should resist pressures to include gene therapy (or any other) research to "round out" their programs or compete with other Institutes. Institute Directors should take the lead, where it seems appropriate, to focus efforts on research in gene discovery, diagnosis or disease pathogenesis and await further developments in vector technology before expanding gene therapy programs.

3. The Panel endorses the efforts of the Director of the Clinical Center to develop strategies to maintain the superb clinical base of the NIH Intramural Program.

Selected references for further reading:

Cystic fibrosis:

Grubb, B. R. et al. Inefficient gene transfer by adenovirus vector to cystic fibrosis airway epithelia of mice and human. Nature 371: 802806, 1994.

Knowles, M. R. et al. A double-blind vehicle-controlled study of adenoviral vector mediated gene transfer in the nasal epithelium of patients with cystic fibrosis. New Engl. J. Med. 333: 823831, 1995.

Adenosine deaminase deficiency:

Blaese, R. M. et al. T Lymphocyte-directed gene therapy for ADASCID: Initial trial results after 4 years. Science 270: 475480, 1995.

(For clinical histories of the ADA-deficient patients in this study, see Hershfield, M. S., Chaffee, S., and Sorensen, R. U. Enzyme replacement therapy with polyethylene glycoladenosine deaminase in adenosine deaminase deficiency: overview and case reports of three patients, including two now receiving gene therapy. Ped. Res. 33: S42S48, 1993.)

Bordignon, C. et al. Gene therapy in peripheral blood lymphocytes and bone marrow for ADA-immunodeficient patients. Science 270: 470475, 1995.

Kohn, D. B. et al. Engraftment of gene-modified umbilical cord blood cells in neonates with adenosine deaminase deficiency. Nature Med. 1: 10171023, 1995.

Hypercholesterolemia:

Grossman, M. et al. Successful *ex vivo* gene therapy directed to liver in a patient with familial hypercholesterolaemia. Nature Genet. 6: 337340, 1994.

(also see Brown, M. S. et al. Gene therapy for cholesterol. Nature Genet. 7: 349350, 1994.)

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Marker, DNA vaccination, and other studies:

Rosenberg, S. A. et al. Gene transfer into humans-immunotherapy of patients with advanced melanoma, using tumor-infiltrating modified by retroviral gene transduction. New Engl. J. Med. 323: 570578, 1990.

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Rooney, C. M. et al. Use of gene-modified virus-specific T lymphocytes to control Epstein-Barr-virus-related lymphoproliferation. Lancet 345: 913, 1995.

Nabel, G. J. et al. Direct gene transfer with DNA-liposome complexes in melanoma: expression, biologic activity,and lack of toxicity in humans. Proc. Natl. Acad. Sci. (USA) 90: 1130711311, 1993.

Dranoff, G. et al. Vaccination with irradiated tumor cells engineered to secrete murine granulocyte-macrophage colony-stimulating factor stimulates potent, specific, and longlasting anti-tumor immunity. Proc. Natl. Acad. Sci. (USA) 90: 35393543, 1993.

Ulmer, J. B. et al. Heterologous protection against influenza by injection of DNA encoding a viral protein. Science 259: 17451749, 1993.

Recent review articles:

Friedmann, T. The promise and overpromise of human gene therapy. Gene Ther. 1: 217218, 1994.

Yu, M., Poeschla, E., and Wong-Staal, F. Progress towards gene therapy for HIV infection. Gene Therapy 1: 1326, 1994.

Table 1. Vector systems in use or under consideration for gene therapy

System	Advantages	Disadvantages	Accumulated Experience	Current or Projected Application

Retrovirus	Efficient entry. Efficient, predictable, and stable integration into host cells. Biology is well understood. Slight immunogenicity*. No viral genes in vector.	Low titer. Limited insert size. Infection limited to dividing cells. Expression difficult to control and stabilize. Potential for genetic damage*. Expensive, complex to prepare and validate.	Extensive	Marker studies. ex vivo treatments, particularly for AIDS and cancer. Vaccines.
Adenovirus	Efficient entry into most or all cell types. High titers. High level of expression. (In principle) no integration of DNA*. Can infect stationary cells.	Vectors contain many viral genes. Highly immunogenic, stimulating both B and T cell responses. Unsuitable for stem cells. Factors controlling tropism poorly understood. Generation of replication competent virus.	Moderate	Localized in vivo treatments: cystic fibrosis, muscular dystrophy, cancer.
Adeno-Associated Virus	Integration at specific sites*.	Requires replicating adenovirus to grow. No helper cell line. Specific integration probably does not occur in absence of viral genes. Very limited insert size.	Moderate	Similar to adenovirus.
Herpesvirus	High titers. Neurotropic*.	Complex construction. No packaging cell lines.	Slight	Neurologic disorders.
Poxviruses	High titers. Large insert size. High expression.	Highly immunogenic. Similar to adenovirus and herpesvirus.	Moderate	Localized, transient in vivo treatment.
Naked DNA	Easy to prepare in quantity. High level of safety*. Virtually unlimited size. No extraneous genes or proteins to induce immune response. Lack of integration*.	Very inefficient entry, uptake into nucleus. No mechanism for persistence or stability.	Moderate	Topical applications, mechanical and accessible (skin, vascular, pulmonary, endothelial cells).

Facilitated DNA (e.g., liposomes)	Same as DNA. More efficient uptake than DNA. Protected from in vivo Targetable to specific cell types*.	Targeting not yet achieved. No mechanism for persistence or stability. Inefficient entry.	Slight	As for naked DNA.

* Denotes theoretical advantage or concern, but one that has not yet been adequately tested.

Table 2. Delivery Vehicle of Clinical Gene Transfer Studies

System	# of Protocols	Percentage
Retrovirus vectors	76	71.7
Adenovirus	15	14.2
Adeno-associated viruses	1	0.9
Cationic liposome complex	12	11.3
Plasmid DNA	2	1.9

Table 3. Categories of Clinical Gene Transfer Protocols

Categ ry	Disease/Disorder	# of Protocols	Percentage
Inherited Monogenic Disorders	**T tal**	**20**	**18.9**
	ADA deficiency	1	0.9
	Alpha-1-antitrypsin	1	0.9
	Chronic granulomatous disease	1	0.9
	Cystic fibrosis	11	10.4
	Familial-hypercholesterolemia	1	0.9
	Fanconi anemia	1	0.9
	Gaucher disease	3	2.8
	Hunter syndrome	1	0.9
Infectious Diseases	**Total**	**8**	**7.5**
	Human inmunodeficiency virus-1	8	7.5
Acquired Disorders	**Total**	**2**	1.9
	Peripheral artery disease	1	0.9
	Rheumatoid arthritis	1	0.9
Cancer (byapproach)	**Total** Antisense	**51**	**49.1**
	Chemoprotection	2	1.9
	Immunotherapy/ex vivo	4	3.8
	Immunotherapy/in vivo	23	21.7
	Pro-drug/HSV-TK/ganciclovir	7	6.6
		11	10.4
	Tumor suppressor gene	4	3.8
Marking Protocols		**25**	**23.6**
All Studies		**106**	**100.0**

Data from Debra J. Wilson, Executive Secretary, Subcommittee on Data Management, Office of Recombinant DNA Activities, NIH

Appendix A

Panel to Assess the NIH Investment in Research on Gene Therapy
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Appendix B

REPORT OF THE FIRST MEETING, MAY 15-16, 1995

With Dr. Stuart H. Orkin and Dr. Arno G. Motulsky serving as cochairs, the Panel to Assess the NIH Investment in Research on Gene Therapy convened for its first meeting on May 15-16, 1995, at the National Institutes of Health (NIH), Natcher Building, 9000 Rockville Pike, Bethesda, MD 20892. During the course of the twoday meeting, panel members heard from Dr. Harold Varmus, NIH Director, and more than 20 additional NIH representatives. Dr. Varmus delineated the panel's mandate, and other NIH staff members described current extramural and intramural programs supporting or otherwise affecting research on gene therapy.

Panel Mandate-Dr. Harold Varmus, NIH Director

Despite many challenges since the first gene transfer experiments were undertaken in microorganisms, biomedical researchers have made considerable progress toward realizing genebased therapies for human disease. Although clinical application of this emerging technology is still in an early phase of development, since 1988 the NIH Recombinant DNA Advisory Committee (RAC) has approved more than 100 protocols that involve tests of gene transfer or putative gene therapy procedures in clinical

settings. Another panel, the Ad Hoc Review Committee of the RAC, which is chaired by Inder Verma of the Salk Institute, is examining how RAC functions in its role as reviewer of proposals to conduct clinical trials involving such gene transfers.

In the aggregate, NIH invests nearly $200 million annually in programs supporting and overseeing gene therapy research. Despite enthusiastic interest and early signs of safety and biological feasibility, however, evidence for therapeutic benefit to patients is meager. Moreover, opinions vary as to what gene delivery systems will prove effective over the long term, and there are unsettled questions as to which diseases are appropriate targets for gene therapy during this phase of its development.

The mandate for the Panel to Assess the NIH Investment in Research on Gene Therapy is to review broadly the gene therapy research enterprise, considering (i) current and proposed investments by NIH centers and institutes in gene therapy and related disciplines, (ii) developments affecting gene therapy in the wider community of academic, government, and industrial laboratories, and (iii) evaluation of the NIH investment in the context of other support for gene therapy research, particularly from the U.S. biotechnology industry and also from outside the United States.

From this comprehensive review, the panel is expected to devise a set of recommendations on NIHsponsored gene therapy research-not a rigid plan-to be presented at the meeting of the Advisory Committee to the Director, NIH, in December 1995. The recommendations are expected to help in NIH budget and program planning for FY 1997 (and, to a limited extent, FY 1996) by addressing specific questions, including the following:

> How should funds and efforts be distributed among areas such as gene delivery system development, gene expression, biology of target cells, pathophysiology, and animal models of disease?
>
> What diseases and organ system targets should be emphasized during this period of gene therapy's development?
>
> What funding mechanisms will be most effective to meet specific program needs? What should be the roles of Requests for Applications (RFAs); centers; the NIH intramural program; pilot production facilities for developing and handling genes, vectors, and target cells; and training programs?
>
> How should NIH deal with policy issues such as patents and licenses, and what are the needs for public and professional education on the science and ethics of gene therapy?

The panel is also encouraged to make additional recommendations on how NIH might coordinate interdisciplinary gene therapy-related activities. For example, should NIH consider setting up a central coordinating office for such research? Moreover, the panel should also examine the impediments to progress in this field. In a broader context, panel members are reminded that the overall NIH budget is not likely to grow but is more likely to stay flat or be reduced in the near future. Hence, if increases in gene therapy research are deemed valuable and necessary, they will necessarily come at the expense of other programs.

NIH Staff Presentations

More than 20 NIH staff members presented information to the panel describing extramural and intramural programs that support or are otherwise relevant to the conduct of gene therapy research. These presentations ranged widely and included descriptions of major and more modest basic and clinical research programs being supported by several institutes and centers, information about grant and contract support mechanisms that may be applicable to future extramural gene therapy programs, available oncampus facilities and current research programs, plans to support a new vector and gene delivery development program, RAC's procedures for conducting reviews of clinical protocols and its experience developing a database for gene transfer clinical trials now under way, and current U.S. patent and licensing policies affecting research in this field.

The National Heart, Lung, and Blood Institute (NHLBI) ($53 million); the National Cancer Institute (NCI) ($10 million); the National Institute of Allergy and Infectious Diseases (NIAID) ($16 million); and the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) support the largest efforts in gene therapy research, with seven other institutes sponsoring smaller programs. In addition, the National Center for Human Genome Research, in cooperation with researchers from several other institutes, is developing basic and clinical research projects strictly as part of its intramural program.

The NIH intramural program, from which the first several clinical protocols to be approved arose, continues to have a strong focus on gene therapy research. The wide variety of projects on the NIH campus to study disparate diseases, particularly rare disorders; specialized facilities, including stateoftheart human stem cell processing and transfer technology; an emphasis on highrisk, lab bench-to-bedside research at the clinical center; a concerted effort to reinvigorate the intramural program that features stringent staff reviews and a new tenure track system; and recently mandated incentives to encourage technology transfer from federal laboratories to the private sector are some of the reasons behind this focus. Recently, some 100 researchers in the intramural program formed a campuswide interest group.

A variety of funding mechanisms is available for supporting gene therapy efforts through the NIH extramural program. Researchers may submit investigatorinitiated grant applications, usually R01s, or prepare applications in response to RFAs, which invite investigators to submit proposals for projects in NIHspecified research areas. Typically, NIH commits funds for RFAs that it issues, and applications receive special reviews. Nonetheless, RFAs allow considerable latitude for researchers at different institutions to establish innovative arrangements and to set up collaborative networks.

In addition, there is a more formal grant mechanism for forming specialized multidisciplinary research centers at single institutions or among several institutions in a "Centers without Walls" program. Besides these grant mechanisms, the extramural program also can designate areas for competitive proposals to do contract research and development projects, usually with very specific targets. Beyond these standard funding measures, the NIH Director now has discretionary authority to transfer 1 percent of NIH funds for a particular fiscal year into research areas of special interest or need.

Additional research resources supported by the extramural program of the National Center for Research Resources (NCRR) are part of a nationwide research infrastructure that already supports some gene therapy research activities and could be tailored or expanded to support additional efforts. For example, 14 of 75 general clinical research centers, most associated with U.S. medical schools, are conducting gene transfer trials. A biotechnology resource center now at Louisiana State University maintains an extensive, everexpanding database for human genemapping studies. There are seven regional primate research centers where gene therapy animal model studies can be conducted. As part of a new resource,

three Institutes (NCI, NHLBI, and NIDDK) will begin supporting in mid1995 one to three national gene vector laboratories, whose establishment is based on a $3.5 million setaside for a joint RFA.

Another important element of NIH's overall involvement in gene therapy research is the role it plays in overseeing policy matters such as the review of clinical protocols. As of May 1995, RAC has recommended approval for 105 human gene transfer protocols, including 77 involving some form of cancer, 19 involving various genetic disorders, and 8 on AIDS. Of this total, 25 are genemarking experiments without any direct therapeutic potential. RAC is now streamlining its review procedures, and full responsibility for several categories of review now resides with FDA.

The NIH Office of Technology Transfer (OTT) serves under a congressional mandate to evaluate research and technology supported by the intramural program and to take appropriate steps to ensure that such intellectual property is further developed. Thus, OTT helps in identifying patentable inventions and filing applications, coordinating the development of cooperative research and development agreements (CRADAs) and material transfer agreements with researchers in industry or at universities, and arranging licensing agreements with industrial partners that seek to develop commercial products. NIH researchers, primarily from NCI and NHLBI, have filed 81 gene therapy-related patent applications (some of them diagnostic developments and others research tools). To date, NIH has completed 22 licenses covering gene therapy-related technologies.

Panel Deliberations

Panel members began to identify problems to address and their general approach for using the next two panel meetings. In general, the panel agreed to invite a total of 12-15 expert speakers to the two meetings, one to be held in Bethesda, Maryland, in July and the other in San Francisco, California, in August. Speakers will be asked to address a series of specific scientific issues affecting gene therapy research, including gene expression; stem cell biology; viral vector and other gene delivery systems; clinical disorders that are targets for gene therapy approaches, including cancer, AIDS, and inherited diseases; industry involvement; and patenting issues. Although an effort will be made to split the two meetings thematically, with the first emphasizing basic science and the second emphasizing applied issues, other constraints from scheduling on relatively short notice may override that design.

The invited speakers, who may include leading exponents in this field and critics, will be asked to focus generically on an assigned topic, not merely to provide a summary of an individual's particular experiences relevant to the topic. In addition to presenting a stateoftheart summary on the assigned topic, speakers will be asked to outline major problems or challenges relevant to the topic, including infrastructure and administrative matters, and to propose ways of solving some of those problems and encouraging progress in their particular subject areas. Speakers will also be asked to provide the panel with a brief summary of important points they plan to make.

In addition to making a general plan for the panel's next two meetings, panel members began to identify problems to address as they assess the NIH investment in gene therapy research. One issue that the panel will consider, which is not unique to gene therapy research, is how different NIH institutes and centers divide resources between intramural and extramural programs. On average, the intramural program budget is about 11 percent of the overall NIH budget, but there is considerable variation across specific programs and projects. Historically, the first few gene therapy clinical protocols were undertaken by researchers in the intramural program, and there is continued strong interest in pursuing such developments. Is that an appropriate strategy?

This issue is related to a more general question of how institutes and centers coordinate overlapping programs in gene therapy research both across extramural portfolios and in the intramural program. In practical terms, a question for the panel may be framed as follows: Should several institutes and centers focus on a few seemingly tractable genetic disorders, such as cystic fibrosis and Gaucher's disease, simultaneously supporting relatively comparable research approaches? Or should early efforts be directed more broadly and targeted for a much more diverse set of diseases?

Other issues that the panel may consider include the following:

Should there be a special new study section to deal exclusively with gene therapy research and related scientific issues?

Should NIH efforts to support gene therapy be scaled back rather than accelerated?

Are recent RFAs issued for specialized gene vector laboratories and for gene therapy programs for specific disorders appropriate at this time? What other diseases or technologies would be appropriate subjects for RFAs?

What should be done about closing the information gap between the biomedical research community and the wider group of medical practitioners as well as the general public regarding gene therapy?

Future Meeting

The second meeting of the Panel to Assess the NIH Investment in Research on Gene Therapy is scheduled for July 13-14, 1995, at NIH, and the third meeting is scheduled for August 17-18, 1995, in San Francisco, California.

List of Speakers

Duane F. Alexander, M.D.
Director
National Institute of Child Health and Human Development

Wendy Baldwin, Ph.D.
Deputy Director for Extramural Research
Office of the Director

James F. Battey, Jr., M.D.
Director, Division of Intramural Research
National Institute on Deafness and Other Communication Disorders

Henning Birk dal-Hansen, D.D.S., Ph.D.
Director, Division of Intramural Research
National Institute of Dental Research

Francis S. Collins, M.D., Ph.D.

John I. Gallin, M.D.
Director
Warren Grant Magnuson Clinical Center

Robert A. Goldstein, M.D., Ph.D.
Director
Division of Allergy, Immunology, and Transplantation
National Institute of Allergy and Infectious Diseases

Michael Gottesman, M.D.
Deputy Director for Intramural Research
Office of the Director

Richard J. Hodes, M.D.
Director
National Institute on Aging

Claude Lenfant, M.D.

Director
National Center for Human Genome Research

Karl Csaky, M.D.
Medical Officer
National Eye Institute

Carl Dieffenbach, Ph.D.
Acting Associate Director
Basic Science Program
Division of AIDS
National Institute of Allergy and Infectious Diseases

Judith Fradkin, M.D.
Chief
Endocrine and Metabolic Diseases Program Branch
National Institute of Diabetes and Digestive and Kidney Diseases

Maria Freire, Ph.D.
Director, Office of Technology Transfer
Office of the Director

Judith L. Vaitukaitis, M.D.
Director
National Center for Research Resources

Robert E. Wittes, M.D.
Acting Director
Division of Cancer Treatment
National Cancer Institute

Director
National Heart, Lung, and Blood Institute

Michael Lockshin, M.D.
Acting Director
National Institute of Arthritis and Musculoskeletal Diseases

Harry L. Malech, M.D.
Deputy Chief
Laboratory of Host Defenses
National Institute of Allergy and Infectious Diseases

Daniel Rotrosen, M.D.
Chief, Host Defense & Inflammation
Division of Allergy Immunology & Transplantation
National Institute of Allergy and Infectious Diseases

Giovanna Spinella, M.D.
Health Scientist Administrator
Developmental Neurology Branch
Division of Convulsive, Developmental and Neuromuscular Disorders
National Institute of Neurological Disorders and Stroke

Harold Varmus, M.D.
Director
National Institutes of Health

Nelson A. Wivel, M.D.
Director
Office of Recombinant DNA Activities

REPORT OF THE SECOND MEETING, JULY 13-14, 1995

With Dr. Stuart H. Orkin and Dr. Arno G. Motulsky serving as co-chairs, the Panel to Assess the NIH Investment in Research on Gene Therapy convened for its second meeting on July 13-14, 1995, at the National Institutes of Health (NIH), Building 31, 9000 Rockville Pike, Bethesda, Maryland 20892. During the course of the two-day meeting, panel members heard from representatives from the academic community and the biotechnology industry who are developing gene vectors and working on clinical protocols in the field of gene therapy. In addition, the committee heard a presentation outlining the impact of patenting on this field. The members of the committee also met for several hours in a closed session.

Vectors: Technical Issues

Initially, researchers have concentrated on developing viruses to serve as vectors for experimental gene

transfer and potential gene therapy procedures. Several types of viruses are being studied for this purpose, with most efforts focusing almost exclusively on retroviruses. Several other types of virus, including adenovirus (AV), adeno-associated virus (AAV), herpesvirus, and human immunodeficiency virus (HIV), are currently also being developed or at least considered for this purpose. In addition, some research groups are studying non-viral vectors, such as liposomes, cationic detergents, and other chemical ligands, for complexing and carrying DNA molecules into target cells.

Several experts believe that, eventually, these two separate vector strategies may converge as researchers try to develop synthetic or semi-synthetic vectors that incorporate the useful features of viruses and chemical agents. Meanwhile, although specific strategies to build useful vectors have strong advocates, no particular vector has emerged as a clear front runner. Each approach has its own problems, and most of them also share problems.

For example, except for AAV, these virus vectors integrate randomly, if at all, in the host cell's chromosomes. Moreover, transduction efficiencies for the virus vectors vary widely--in part reflecting their poor ability to integrate into the chromosomes of resting cells. This problem may even affect HIV, despite a widely held notion that it can infect resting cells. Nonetheless, according to Dr. Richard Mulligan, some of the more recently refined retroviral vectors efficiently transduce non-resting target cells, particularly if they carry appropriate LTR sequences and selectable marker genes or, in some cases, specific promoter-enhancer sequences.

Another general problem is that very little research has been done to incorporate externally controllable gene sequences into viral vectors. For instance, regulated beta-globin gene expression is perhaps the most widely studied prototype. However, when this gene is transduced successfully into human cells growing in tissue culture, its expression cannot yet be properly regulated. Some of these difficulties in attaining gene regulation may arise because of the randomness of integration.

In part because gene regulation questions are unanswered, determining the appropriate dosage levels for viral vectors presents another major challenge. For example, according to Dr. Alan Smith, in clinical trials involving patients with cystic fibrosis (CF), there is a concern that the vector and the CFTR gene product it carries may pose problems if they are delivered in too high doses. Because CFTR is ordinarily effective in cells when present at very low levels, low doses of the transferred gene may be required for effectiveness and may be less likely to induce host inflammatory responses.

These considerations raise a more general and potentially serious problem, namely that viral vectors may carry genes--either their own or the particular recombinant genes they are modified to carry--that elicit host immune system responses. This phenomenon might interfere with the efficacy of gene therapy procedures, possibly curtailing long-term expression of transferred genes and prohibiting repeat administration of the therapeutic agent. Other factors, such as counter selection of the transduced cell by immune or other mechanisms and the randomness of integration, may also contribute to apparent low transduction efficiencies and/or short-lived expression of transferred genes.

Dr. Smith said that cationic lipid vectors are being improved and now perform as much as 500-fold more effectively than naked DNA but are still less effective than is the AV vector in rodent model systems. A potential advantage of cationic lipids is that they can be administered repeatedly to rodents. However, at high doses they induce some focal inflammatory responses, albeit without evidence of eliciting antibodies or provoking T cell activation. Dr. Smith speculated that cationic lipids activate macrophage cells.

Additional advantages and problems associated with specific vector candidates:

Retr viral Vectors Although retroviral genes have been extensively modified to ensure that these vectors cannot replicate and are unlikely to recombine, this extensive modification makes them that more difficult to produce. For example, sometimes several packaging cell lines are needed to produce the vectors, and these cell lines are difficult to derive and maintain. Integration of retroviral vectors into the host chromosome is random, and expression levels of the transgene vary and often are unacceptably low.

In addition, host cell range tends to be narrow, although introduction of genes from other viruses such as vesicular stomatitis virus (VSV) may help in broadening that range. However, the presence of VSV genes may introduce new toxicity problems, leading to damage or killing of the host cell.

Adenovirus (AV) Several research groups are investigating whether systematic removal or modification of AV genes can reduce host flammatory responses when this virus serves as a gene vector.

Dr. James Wilson said that other approaches to controlling the inflammatory response are being considered, including production of antibodies to block T cell activation, use of agents such as the drug cytoxan to block T cell proliferation, and use of cytokines to reduce or block production of neutralizing antibodies.

Dr. Thomas Shenk said that several AV genes influence tumor formation in animal model systems and malignant transformation of cultured cells. Thus, AV represents a potential problem when modified versions of the virus are used as vectors, even though AV has not been observed to cause human tumors. He also is studying the molecular and cellular events required for AV to recognize, bind to, and penetrate target cells, and to deliver and integrate the genes it carries to the target cell nucleus.

- **Adeno-Associated Virus (AAV)** AAV, when modified to serve as a vector, lacks certain control sequences and has limited DNA (4.4 kb) carrying capacity, according to Dr. Kenneth Berns. Moreover, he pointed out that the virus is difficult to produce in high titers and needs to be purified in cesium chloride gradients, a laborious procedure. Because AAV integration is site specific, at least in the wild type, there is a question whether repeat dosing with this vector will be possible because follow-up doses may be routinely excluded from the AAV-occupied site on the host chromosome. In some researchers' hands, AAV has a very low transduction efficiency unless AV or AV genes are also present.

Clinical and Animal Model Studies: Technical Issues

Invited speakers described gene therapy clinical trials involving a range of diseases, including inherited conditions such as adenosine deaminase (ADA) deficiency and cystic fibrosis (CF), a range of malignancies, and AIDS. Some of the justification for conducting clinical trials at this relatively early stage of gene therapy's development is that other well-tried approaches have not yielded satisfactory therapies for treating these usually deadly diseases. Another problem, cited frequently in the case of CF and applicable to several other cases, is that animal model systems are far from perfect, sometimes making results from gene transfer experiments incomplete or misleading.

Yet another set of problems entails uncertainties over the target cells for gene transfer procedures. Dr. Arthur Nienhuis noted that several issues may help to account for low overall gene transfer efficiency in clinical settings. These include the phase of the cell growth cycle that a particular target stem cell may be in, the current unavailability of effective cytokines to regulate that cycle, difficulties in stimulating specific viral receptor production by the cell, and problems in improving the transduction efficiency of target cells. Stimulation with cytokines or, alternatively, the introduction of drug resistance markers and subsequent use of the corresponding drug may provide ways of expanding specific transduced target cell populations. However, Dr. Nienhuis cautioned that such approaches are still at a very early, preclinical stage of development.

Results from clinical trials so far are limited. Relatively few patients have been treated; measures of biological response are often not adequately sensitive, except in cases where host inflammatory responses have been reported; the effects observed seem to be erratic; and the reporting of effects so far has been almost entirely anecdotal, rather than in peer reviewed publications.

According to Dr. Ronald Crystal, AV-delivered CFTR genes may be expressed along airways of CF patients as many as four days after being administered; however, that expression is observed in only a low percentage of the patients treated. According to Dr. James Wilson, in other experiments involving CF patients, expression of the CFTR gene is rare, not stable, but also not toxic. Although sustained expression is attained in knock-out mice, efforts to introduce the CFTR gene in other animal model systems tend to induce immune responses directed to vector (AV) genes.

Clinical results are also variable in the few ADA patients who are partaking in gene transfer experiments, according to Dr. Michael Blaese. One youngster has been infused 11 times over 23 months with her own T cells after they were treated with a retrovirus carrying an ADA gene, and ADA+ T cells have persisted for two years following the eleventh infusion. He said there is one copy of vector per peripheral T cell, and a positive signal for circulating mRNA (earlier, that signal was "intermittent"). A complicating factor is that PEG-ADA is still being administered to the patient, albeit in a low dose that was established before she more than doubled in weight.

The results for a second child under the same treatment regime are more ambiguous but apparently less promising. However, Dr. Blaese said that three other children whose cord blood was treated at birth show persistent expression of the vector after more than 12 months following the procedure. In addition, good expression of the ADA retroviral-delivered gene was obtained in vitro from foreskin cells obtained from two of these patients, suggesting that small skin grafts using modified cells might be an effective alternative means of delivering the corrective ADA (or other) genes.

Results from gene transfer experiments involving AIDS or cancer patients are scanty. For example, in some cases the HIV+ member of an identical twin pair develops positive skin responses following a gene transfer procedure, but whether this change will lead to clinical benefits is not yet known.

Dr. Philip Greenberg also refers to "transient" antiviral effects and "proof of concept" in gene transfer experiments involving modified HIV genes in patients with AIDS.

A wide range of clinical experiments involving patients with a variety of cancers is under way. Dr. Blaese said there is some evidence of efficacy, such as tumor shrinkage in patients with glioblastomas. Some of the protocols call for the gene transfer procedure to induce immune system responses against the tumor, according to Dr. Gary Nabel. In some cases, patients appear to go into long-term remission;

in other cases, the effects are transient. Partial effects are commonplace in cancer treatment, and gene therapy approaches therefore may find acceptance as a useful addition to the therapeutic arsenal.

Dr. Nabel and Dr. John Mendelsohn pointed out that, in gene transfer experiments involving cancer patients, better measures of biological activity are needed. This need is particularly acute in early tests involving patients with advanced disease when other treatments and other clinical abnormalities make assessment of a single experimental procedure exceedingly difficult.

Responses to the question of whether the field is ready for clinical trials:

Dr. Mulligan: Too much of current research is "not worth taking to patients." The field needs "wise people to prune and avoid copy cat" projects.

Dr. Smith: "We don't know it won't work." Regarding uncertainties about identifying and successfully targeting epithelial stem cells in human airways, he said that treatments would need to be repeated because cells are expected to turn over every 60-80 days. Also, problems have been seen in animal models where the transgene was expressed in excess; transfection is inconsistent in monkeys when high-dose vectors are tested but successful at low doses in cotton rats; and the goal is not specifically to achieve stem cell integration or to "duplicate" nature but to produce a "useful" therapeutic agent.

Dr. Crystal: Through clinical trials, investigators are "learning how to evaluate" the gene transfer procedures. In the case of trials involving CF patients, currently antibody-based tests are not sensitive enough to detect the product of the transfected CFTR gene; there are other difficulties with PCR-based assays. Non-human primates, such as rhesus monkeys, are not a reliable model for CF.

- Dr. Mendelsohn: Oncologists have taken drug studies as far as seems possible so the "new approach of gene transfer is exciting ... and needs to be backed."

- Dr. Shenk: If gene transfer procedures appear to work in animal models of some diseases, particularly cancer, they are probably ready for clinical trials. For other diseases, such as CF, particular problems with vectors and gene delivery came to light only because of findings from early-stage clinical trials. Sometimes researchers are unaware of a phenomenon until they do clinical trials and would not have known to look for it during animal experiments. Once appreciated, the phenomenon may better be studied in model systems. However, a moratorium on clinical trials is not warranted.

Basic and Clinical Infrastructure and Training Issues

Speakers identified several areas of basic biology research that need greater emphasis:

better understanding of hematopoietic cells and of bone marrow transplantation; stem cell heterogeneity; lung epithelial biology; inflammatory responses; and apoptosis, which may prove important for treating diseases such as cancer and AIDS;

better understanding of basic virology and manipulations needed to improve vectors and their delivery to appropriate cells in target tissues and organs or to tumors; and

better models for preclinical studies of disorders that may be subject to gene therapy approaches; however non-human primate models cannot replace clinical research because they are difficult to develop and costly to use.

Speakers also identified several logistical and pragmatic barriers to overcome to foster progress in gene therapy research:

Means are needed for producing high amounts of vectors of suitable quality for use in small-scale clinical experiments; there is disagreement whether NIH should sponsor GMP vector production facilities.

More sensitive and reliable assays are needed for assessing the biological activity of transferred genes and clinical end points.

Novel relations among government, industry, and academic institutions will be needed at the research level and as novel, clinically useful reagents are developed; more than 50 companies are said to be doing gene therapy-related research.

Industry representatives referred to regulatory impediments and criticized the current clinical protocol review process involving oversight by the NIH RAC and FDA.

Some participants raised the issue of conflicts of interest.

One speaker suggested that more international collaborations should be encouraged.

Several speakers referred to training needs, but there is not full agreement on the kind of training that should be emphasized. In general, participants said they prefer rigorous training in basic scientific disciplines, even for young clinical investigators who want to work in the field of gene therapy. There is some sense that, if gene therapy develops rapidly into a successful clinical modality, new means will be needed to integrate these approaches into the current system for delivering health care, which itself is rapidly changing.

Patent Issues

Because many patent applications pertaining to gene therapy technology are still pending, their impact on this emerging field remains difficult to predict, according to Ms. Rebecca Eisenberg. She recommends that research institutions rely more on non-exclusive licensing agreements as a way of circumventing several potential problems and thereby not hindering the efficient development of this field.

The U.S. Patent and Trademark Office (PTO) has issued several broad-based patents covering fundamental gene therapy technologies, including a patent granted to NIH and licensed exclusively to Gene Therapy, Inc., covering ex vivo gene therapy and another patent granted to the University of Michigan and licensed exclusively to Genovo that covers any viral gene therapy vector carrying the CFTR gene, which is impaired in individuals with CF.

Ms. Eisenberg said that these examples as well as other signs indicate this field of biotechnology is likely to be "more littered" with patents than is the earlier emerging field of biotechnology involving the

discovery and development of therapeutic proteins.

Ms. Eisenberg attributes this difference to the fact that universities and other research institutions are being even more aggressive now than a few years ago in pursuing patent protection for intellectual property their researchers are developing. The Bayh-Dole Act, which specifies that such institutions may retain ownership in patents arising from federally sponsored research, now provides strong incentives for pursuing patents--raising expectations in the university community that royalties from licensing agreements eventually will become a significant source of revenue.

Although in some noteworthy cases involving biotechnology inventions universities are benefitting from significant royalty payments, there are potential problems to face from the flurry of patent applications being put forth in the field of gene therapy, according to Ms. Eisenberg. Perhaps chief among them is that research teams and clinicians may, in effect, be faced with a series of "toll booths" along the road to developing and implementing effective gene therapy procedures. She says that research groups may be hemmed in and financially pinched if they have to enter into complex cross-licensing agreements or if institutions set royalty requirements at levels that are too high. Additional complications include potential priority disputes between competing "inventors," disagreements over ownership when researchers at several institutions are collaborating on a project, and differences arising because some researchers such as medical geneticists tend not to patent their work, whereas other researchers such as molecular biologists do so.

Future Meeting

The third meeting of the Panel to Assess the NIH Investment in Research on Gene Therapy is scheduled for August 17-18, 1995, in San Francisco, California.

List of Speakers

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REPORT OF THE THIRD MEETING, AUGUST 17-18, 1995

With Dr. Stuart H. Orkin and Dr. Arno G. Motulsky serving as co-chairs, the Panel to Assess the NIH Investment in Research on Gene Therapy convened for its third meeting on August 17-18, 1995, at the Sir Francis Drake Hotel, San Francisco, California. During the first day of the two-day meeting, panel members heard from several representatives of the academic community and the biotechnology industry who are developing gene vectors and working on clinical protocols in the field of gene therapy. The panel members also heard from researchers outside this field who are working at a more basic level. Some of these researchers are skeptical about certain developments in gene therapy, calling some of them misguided, others premature. On the second day, the members of the committee met in a closed session to outline the report they plan to deliver to NIH Director Harold Varmus.

The Case for Re-Emphasizing Basic Research

Several investigators who appeared before the panel made a case for re-emphasizing basic research and pursuing other strategies for treating some of the diseases that researchers in the field of gene therapy have been studying. One line of argument is that alternative biochemical manipulations appear simpler to apply than gene transfer techniques and might reach fruition sooner. Another line of argument is that gene transfer approaches are premature because not enough is understood in the field of stem cell biology, a vital prerequisite for success in gene therapy.

Some of these investigators criticized current proponents of gene therapy for portraying the field in unrealistic terms and misrepresenting progress as more rapid than it has been. For example, Dr. Joseph Goldstein called for greater realism in the way these researchers present views of their field to the public. He also pointed out that the development of any new therapeutic product is a laborious, time-consuming effort.

Dr. Goldstein said that some of the diseases now targeted by gene therapy researchers might be treated sooner, by other strategies, if investigators pursued more traditional studies into the pathophysiologic basis of the diseases in question. He cited several examples where this alternative approach has paid off either recently or several decades ago. For instance, prednisone treatment reverses steps in a defective sterol metabolic pathway that otherwise leads to masculinization. In a more recent development, an inhibitor of cholesterol production (lovastatin) overcomes a LDL receptor deficiency and, by lowering cholesterol levels, helps to prevent coronary heart disease.

Dr. Goldstein also referred to several genetic diseases that arise because of protein trafficking abnormalities. In some of those cases, the critical mutations lie outside the functional coding region of the enzyme product and, instead, serve to misdirect nascent proteins, which are transported into the wrong biological compartments. He called for basic research that could provide an alternative means to gene therapy for correcting such defects.

Dr. Irving Weissman and Dr. Goldstein said that studies with animal models deserve greater emphasis than they are receiving by researchers who are moving quickly from basic research to the clinic to test new ideas about gene therapy. This general problem is particularly applicable to several unsolved problems involving stem cells, which are important but elusive targets of many gene transfer protocols in which long-term gene expression is a major goal.

Dr. Weissman pointed out that stem cell biology in humans and mice is essentially equivalent. From studies on mice, investigators have learned that there are three critical subsets of stem cells in bone marrow and that the most desirable subset for gene transfer is the rarest and is very difficult to work with.

A key problem in the use of retroviral vectors is to determine which factors will induce self-renewing stem cells to divide. Without such detailed information that can be applied practically, gene transfer procedures will likely fail because genes will not be integrating into target progenitor cells. Dr. Weissman said that, with such fundamental obstacles to human gene transfers, it may make sense to focus instead on activating genes that are already present rather than on replacing defective or missing genes.

Dr. Victor Dzau pointed out that, for certain clinical conditions including several that affect the cardiovascular system, short-term rather than long-term gene expression may be all that is needed to address specific problems. Moreover, in a rabbit model system, studies indicate that localized high pressure can improve DNA transduction rates, enabling antisense oligonucleotides to block transiently a cell-proliferative response that otherwise may interfere with surgically grafted blood vessels. Experiments indicate that high pressure also enhances the delivery of oligonucleotides into cultured human cells, improving the efficiency of transduction.

Dr. Gerald Crabtree described the use of synthetic, lipid-soluble dimerizing reagents that can be used to bring cellular regulatory proteins into covalent juxtaposition, thereby changing their functional status. For example, with appropriate dimeric reagents, specific transcriptional factors might be modified in such a way that they permanently activate this process, meaning that a transgenic cell produces high levels of the designated gene product. Another potential use of such dimerizing reagents would be to cross-link specific cell receptors to induce apoptosis. Although this approach shows promise and many other applications are imaginable, studies are limited so far to cellular systems and considerable work will be needed before animal model studies can be undertaken.

The Case for Simultaneous Basic and Clinical Research

Several investigators who came before the panel said that the rapid movement from the laboratory to the clinic to test gene transfer protocols sometimes is essential. Dr. W. French Anderson said that, with more than 120 clinical protocols now approved, the nearly five-year-old field of gene therapy research is showing healthy progress. He also predicted that it will be 15 to 20 years before the full potential of current research will be realized.

Dr. Flossie Wong-Staal pointed out that in vitro studies or animal models of AIDS are far from adequate, making it best to go forward rapidly with small, focused clinical trials to test gene transfer procedures. Although the rationale for using ribozyme genes to block HIV gene expression appears sound when tested at the cellular level, many questions, such as the extent to which target cells in patients will be genetically modified and then selected and whether HIV will develop resistance to the ribozyme, can only be addressed through clinical studies.

Dr. Anderson outlined a variety of gene therapy research studies at his institution, suggesting that this locally concentrated diversity of interests and ideas is another sign that this field is healthy and populated with creative young investigators. He also described a long-term project that involves making a series of improvements in a current retroviral-based vector that could extend its half-life in the host circulatory system, increase its efficiency of binding to and entering specific target cells of the host, improve its chances of delivering genes for long-term expression, and eventually lead to a readily injectable gene-delivery product. Efforts to realize these goals are only at the "very beginning."

Other current basic research developments may eventually help solve some of the challenges that investigators conducting human gene transfer protocols now face. For example, Dr. Donald Kohn described efforts to modify the long terminal repeat (LTR) in a retroviral vector now being used in gene transfer protocols as a way of extending the expression of transferred genes after they are delivered to target cells. Hematopoietic cells from mice are providing a valuable model in which to study this problem, and some results indicate that methylation within the LTR correlates with the disappearance of transferred gene expression.

In a model system in which human bone marrow cells are introduced into immunologically deficient nude mice, Dr. Kohn and his collaborators find that the addition of stroma enhances gene transfer in vitro and also extends long-term expression of the transduced genes. The impact of growth factors on these steps is also being evaluated. Dr. Kohn said that, despite the value of this information from experiments in mice, clinical trials are needed to understand in detail how each of these steps work in humans.

One important problem that has come to light from early gene transfer clinical studies is that host immune responses may abbreviate expression of transferred genes. Dr. Paul Tolstoshev described efforts to develop sophisticated vectors that can overcome this problem. Less immunogenic vectors are being constructed for use in conjunction with immunosuppressive agents such as dexamethasone or cyclosporin that can reduce immune system responses, including deleterious inflammatory reactions.

Academic, Industry Representatives' Comments on Policy Questions

Industry and academic representatives said that clinical trials are an important element of gene therapy, providing data that have helped in choosing among models and in other ways are proving essential for the development of this field. Dr. Wong-Staal said that the cost as well as the complexity of current regulatory requirements impose barriers on efforts to design and conduct small-scale clinical trials. Moreover, simplifying annual reporting requirements would be helpful to investigators.

Dr. Anderson pointed out that progress is more likely to be rapid if individual investigators--rather than a central committee--direct research decision making. He also recommended that the development and use of vectors made in NIH-supported specialized laboratories not be restricted to only those researchers whose work is being supported by NIH. He was less certain whether a policy of limiting such vector

development to research on orphan diseases should be adopted.

Dr. Barrie Carter pointed out that efforts to begin the first clinical trials and subsequent efforts to test additional gene transfer protocols in clinical settings are driving a great deal of basic research in biology. Although NIH programs provided the fundamental research from which gene therapy derives, industry now furnishes enormous resources to further these developments. He noted that NIH spends about $200 million annually on gene therapy research, and this amount represents less than 2 percent of total NIH research expenditures. He recommended that NIH spending be maintained at this level, concentrating in several program areas such as gene delivery systems, target cell biology, and preclinical models.

Dr. Carter noted that basic and clinical research within the NIH Intramural Program is a valuable component of overall efforts in the field of gene therapy. He also praised the role NIH plays in supporting programs in basic research on viral vectors and at General Clinical Research Centers. However, he questioned the value of NIH setting up new gene vector production facilities, suggesting that industry can do a better job producing vectors. Dr. Tolstoshev noted that companies are conducting a great deal of fundamental research on gene vectors and, in many cases, these vectors are being made available to university researchers for testing and evaluation.

Industry representatives pointed to several technology transfer arrangements that are helpful to them, despite specific obstacles which sometimes arise. For example, Cooperative Research and Development Agreements (CRADAs) are now being used extensively to establish relationships between companies and NIH investigators in the field of gene therapy. Dr. Tolstoshev said that the many CRADAs established between his company, Genetic Therapy, Inc. (GTI), and individual NIH investigators are particularly helpful in leveraging the company's expertise. Other types of agreements, including material transfer agreements and scientific collaborations between industry and university researchers, are providing a major source of funding for this developing field, and that source could grow larger as major established pharmaceutical companies take a greater interest in gene therapy.

Legal and policy difficulties sometimes have made CRADA negotiations drawn out and cumbersome. Dr. Tolstoshev noted that, by eliminating a clause calling for "reasonable pricing" of drugs and other products that may flow from a CRADA, NIH removed what had become an important stumbling block for industry. However, he also said that protracted negotiation of the legal terms of many CRADAs can still be an impediment to efficient technology transfer.

Industry representatives pointed to other important policy issues, including a need for clear-cut patenting policies and the relative value of exclusive versus non-exclusive licensing agreements. Industry representatives said that, in general, licensing agreements granting particular companies the exclusive right to commercialize intellectual property developed by NIH investigators are more likely to provide essential incentives to pursue development than are non-exclusive agreements.

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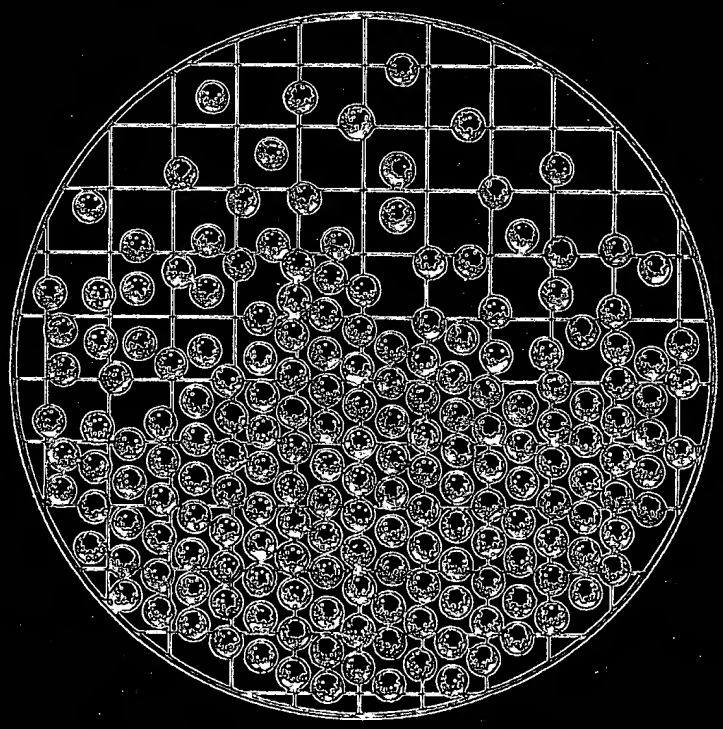

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defective, the host DNA is degraded and the adenovirus cytopathology is augmented (408).

The E1B large polypeptide (58 kd) in the nomenclature of Sarnow et al. binds to a 53-kd cellular protein that is increased in amount in many different cells transformed by unrelated DNA tumor viruses (336). For example, in SV40-transformed cells, the 53-kd cell protein binds to the SV40 large-T antigen, suggesting that the 53-kd protein may be involved in the transformation. Monoclonal antibody, induced by injections of the Ad 5 E1B 58-kd tumor antigen, immunoprecipitated the 53-kd cell protein in complexes with the -kd polypeptide but was not reactive with the 53-kd polypeptide alone (240). For Ad 12-transformed cells, a physical association between the E1B 55-kd protein (analogous to the Ad5 58 kd) was not demonstrated, but the 55-kd protein appears to regulate the levels of the 53-kd protein induced after adenovirus transformation. It has recently been suggested that the 53-kd protein is an anti-oncogene similar to Rb, the retinoblastoma gene (96,154). The 53-kd protein found in large amounts in transformed cells, has a point mutation that differentiates it from the normal homologue found in lower amounts in normal tissue. The 53-kd point mutant protein appears in both transformed and "normal" cells in tissue culture, which suggests that its presence may be necessary in order to maintain the viability of all cells in culture. In addition to the interactions of the E1B 58-kd proteins just described, this same protein also interacts with an early region 4, 25-kd protein in productively infected cells (65). Each of the two proteins is necessary for progression of the lytic cycle to the efficient production of viral DNA, the expression of late viral genes, and host-cell shutoff (139). Mutants in either the E1B 58-kd or the E4 25-kd protein have the same defective phenotype during the lytic cycle. The size of these two proteins has recently been revised (E1B 55 kd and E4 34 kd) based on sequencing data of their open-reading frames (66).

Region E2

E2A codes for a single-strand DNA-binding protein (DBP) that has also been reported to bind to the ends of dsDNA (229) and is made in large amounts in adenovirus-infected cells. The molecular weight estimated by polyacrylamide gels was 72 kd, but recent sequencing of an open-reading frame in the *2A* region of both the Ad 2 and Ad 5 genomes suggested a molecular weight of 58 kd (200,201). Initial isolates of this polypeptide included a 44-kd proteolytic fragment that could be produced intentionally by controlled chymotrypsin digestion of the 72-kd polypeptide (5,191,388). Further proteolytic digestion yielded a 34-kd fragment, which, like the 44-kd fragment, is at the carboxyl terminus of the complete DBP (107). The DBP is heavily phosphorylated *in vivo*, but the purified 34-kd fragment is free of detectable $^{32}PO_4$ label (107,191). Thus all the *in vivo* phosphates cluster at the N-terminus. There are several ts mutants (H5*ts*125 and H5*ts*107) with the same single amino acid change in the sequenced carboxyl terminus (202). These two apparently independent isolates have exactly the same defect in the polypeptide. Second-site ts^+ revertants of the H5*ts*107 mutant have been isolated, and the changes also seem to cluster in very limited regions in multiple, separate isolates (202,269). The Ad DBP is needed for DNA replication both *in vivo* and *in vitro* (107,121,160,180,196,213,390). The N-terminus of DBP is dispensible for DNA replication, but it has recently been shown to be necessary in several situations where it probably functions in the regulation of transcription (5,107,196).

The E2B region codes for two proteins needed for DNA replication (108,224,351). The precursor to the terminal protein (pTP) is made as an 80-kd polypeptide that is cleaved, in the process of viral assembly, to 55 kd while covalently attached to Ad DNA (46). The cleavage is mediated by a 23-kd protease that is coded by the viral genome near coordinate 60 (402). A 140-kd Ad DNA polymerase (Ad DNA Pol) has been shown to map in the E2B region by complementation assays using an *in vitro* DNA synthesis system (108,223,224). Purified wt Ad DNA polymerase complemented the defect in the H5*ts*149 DNA negative mutant at elevated temperatures. The defect in H5*ts*149 has been shown to map between 18 and 22 map units. The functional role of the E2A and E2B polypeptides will be further discussed in the section entitled "Adenovirus DNA Replication."

Regions E3 and E4

The E3 region is nonessential in tissue culture but has probably been maintained in numerous human serotypes studied because of its function in modulating the host response to Ad infection. For example, a 19-kd protein with two to four N-linked glycosylation sites binds to the MHC polypeptide heavy chain in the endoplasmic reticulum (ER) and prevents the transport of MHC polypeptides to the cell surface (34,100,284, 291). The down-regulation of MHC polypeptides on the plasma membrane decreases the target for recognition of infected cell antigens by cytotoxic T lymphocytes. This mechanism to regulate the amount of MHC polypeptide is different from the transcriptional control described for the E1A inhibition of MHC polypeptide in Ad12 transformed cells, but in each case the amount of cell surface MHC polypeptide is decreased.

-continued

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE:

(iii) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

CATCTGAACT CAAAGCGTGG 20

What is claimed is:

1. A recombinant adenovirus which carries an adenovirus vector construct comprising an expression region encoding p53 under the control of a cytomegalovirus immediate early promoter, wherein infection of a tumor cell with said adenovirus results in a p53 protein level sufficient to inhibit tumor cell growth in vivo.

2. The recombinant adenovirus of claim 1, wherein the recombinant adenovirus achieves a p53 protein level in said tumor cell of about 14-times that of endogenous p53 in infected adenovirus H460 cells.

3. The reccombinant adenovirus of claim 1, wherein the recombinant adenovirus achieves a p53 protein level in said tumor cell of about 2- to 4-times that of β-actin in infected adenovirus H358 cells.

4. The recombinant adenovirus of claim 1, wherein said polyadenylation signal is an SV40 polyadenylation signal.

5. An adenovirus vector construct comprising an expression region encoding p53 under the control of a cytomegalovirus immediate early promoter, wherein infection of a tumor cell with said adenovirus results in a p53 protein level sufficient to inhibit tumor cell growth in vivo.

6. The recombinant adenovirus of claim 4, wherein said polyadenylation signal is a protamine polyadenylation signal.

7. The recombinant adenovirus of claim 1, wherein the region encoding p53 replaces E1A and E1B coding regions.

8. The vector construct of claim 1, wherein the vector construct achieves a p53 protein level in said host cell of about 14-times that of endogenous p53 in adenovirus vector construct containing H460 cells.

9. The vector construct of claim 1, wherein the vector construct achieves a p53 protein level in said host cell of about 2- to 4-times that of β-actin in adenovirus vector construct containing H358 cells.

10. The vector construct of claim 8, wherein said promoter is an CMV IE promoter.

11. The recombinant adenovirus of claim 8, wherein said polyadenylation signal is an SV40 polyadenylation signal.

12. A recombinant adenovirus which carries an adenovirus vector construct comprising an expression region encoding p53 under the control of a promoter, wherein said adenovirus expresses p53 in a host cell at a level sufficient to restore growth suppression to a tumor cell in vivo.

13. The recombinant adenovirus of claim 8, wherein said polyadenylation signal is a protamine polyadenylation signal.

14. The vector construct of claim 8, wherein the region encoding p53 replaces E1A and E1B coding regions.

15. A recombinant adenovirus which carries an adenovirus vector construct comprising an expression region encoding p53 under the control of a cytomegalovirus immediate early promoter, wherein said adenovirus expresses p53 in a tumor cell at a level sufficient to restore growth suppression to said tumor cell in vivo.

16. An adenovirus vector construct comprising an expression region encoding p53 under the control of a cytomegalovirus immediate early promoter, wherein said adenovirus vector construct expresses p53 tumor in a cell at a level sufficient to restore growth suppression to said tumor cell in vivo.

17. A recombinant adenovirus which carries an adenovirus vector construct comprising an expression region encoding p53 under the control of a cytomegalovirus immediate early promoter, wherein said adenovirus expresses p53 in a tumor cell at a level sufficient to kill said tumor cell in vivo.

18. An adenovirus vector construct comprising an expression region encoding p53 under the control of a cytomegalovirus immediate early promoter, wherein said adenovirus vector construct expresses p53 in a tumor cell at a level sufficient to kill said tumor cell in vivo.

* * * * *

-continued

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE:

(iii) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

CATCTGAACT CAAAGCGTGG 20

What is claimed is:

1. A DNA segment comprising an adenoviral ITR and a p53 coding region, the coding region positioned under the control of a CMV promoter.

2. The DNA segment of claim 1, further comprising a polyadenylation signal position 3' of the p53 coding region.

3. The DNA segment of claim 1, which does not contain a functional adenoviral E1 coding region.

4. The DNA segment of claim 1, wherein the adenoviral ITR region is a left end ITR.

5. The DNA segment of claim 1, further comprising an E1 enhancer.

6. The DNA segment of claim 1, further comprising from about 0 map units to about 16 map units of the adenoviral genome, excluding from about 1.3 map units to 9.2 map units of the adenoviral genome.

7. The DNA segment of claim 1, wherein the DNA segment is an expression vector.

8. The DNA segment of claim 2, wherein the polyadenylation signal is an SV40 polyadenylation signal.

9. The DNA segment of claim 4, further comprising an E2 adenoviral gene.

10. The DNA segment of claim 9, further comprising an E4 adenoviral gene.

11. The DNA segment of claim 6, further comprising from about 0 map units to about 100 map units of the adenoviral genome, excluding from about 1.3 map units to 9.2 map units of the adenoviral genome.

12. The DNA segment of claim 7, wherein the expression vector is an adenovirus expression vector.

13. The DNA segment of any one of claims 1 through 12, wherein the DNA is packaged in an adenovirus.

* * * * *

-continued

TCGTTTCTCA GCAGCTGTTG 20

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE:

(iii) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

CATCTGAACT CAAAGCGTGG 20

What is claimed is:

1. A method of inducing cell death in human tumor cells comprising administering directly to a tumor comprised of cells which lack functional p53, an adenovirus vector which does not express functional E1B, wherein the vector further comprises and expresses a DNA sequence encoding wild-type p53, and wherein sufficient wild-type p53 is expressed in the tumor cells to induce cell death resulting in regression of the tumor.

2. The method of claim 1, wherein said tumor cell is a human breast cancer cell.

3. The method of claim 1, wherein said tumor cell is an epithelial cancer cell.

4. The method of claim 1, wherein said tumor cell is a human lung cancer cell.

5. The method of claim 4, wherein said lung cancer cell is a small cell lung carcinoma cell.

6. The method of claim 4, wherein said lung cancer cell is a non-small cell lung carcinoma cell.

7. The method of claim 6, wherein said non-small cell lung carcinoma cell is an adenocarcinoma cell.

8. The method of claim 6, wherein said non-small cell lung carcinoma cell is a large-cell undifferentiated cell.

9. The method of claim 6, wherein said non-small cell lung carcinoma cell is a squamous carcinoma cell.

10. The method of claim 1, wherein the vector comprises adenovirus type-5 sequences.

11. The method of claim 1, wherein the promoter is selected from the group consisting of SV40, polyoma and adenovirus 2 promoters.

12. The method of claim 1, wherein the vector further comprises a polyadenylation signal.

13. The method of claim 12, wherein said the polyadenylation signal is from SV40.

14. The method of claim 1, wherein the vector is a replication-defective adenovirus.

15. The method of claim 14, wherein said vector lacks the E1B coding region and the DNA sequence encoding p53 is substituted for therefore.

16. The method of claim 15, wherein said vector lacks map units 1.3–9.2 of adenovirus type-5, and wherein said DNA sequence is substituted therefor.

17. The method of claim 16, wherein said vector is packaged in an E1-expressing cell line.

18. The method of claim 17, wherein said E1-expressing cell line is the 293 cell line.

19. The method of claim 14, wherein expression of the DNA sequence is regulated by the adenovirus E1 enhancer.

20. The method of claim 19, wherein said vector further comprises an origin of replication.

21. The method of claim 20, wherein said origin of replication is viral and is selected from the group consisting of SV40, polyoma, adenovirus, VSV and BPV.

22. The method of claim 21, wherein said origin of replication is from an adenovirus.

23. The method of claim 1, wherein said vector further comprises an enhancer.

24. The method of claim 1, wherein said administration is by direct injection of the tumor.

25. The method of claim 1, wherein said administration is by direct intravenous injection of the tumor.

26. The method of claim 1, wherein said adenovirus composition is administered via intratracheal injection.

27. The method of claim 1, wherein said administration is by infusion over a period of time.

28. The method of claim 27, wherein said period of time is 48 hours.

29. The method of claim 1, wherein about 10^3 to 5×10^{12} adenovirus vector particles are administered.

30. The method of claim 29, wherein about 10^{10} to 5×10^{12} adenovirus vector particles are administered.

31. The method of claim 30, wherein about 10^{10} adenovirus vector particles are administered.

32. The method of claim 30, wherein about 5×10^{12} adenovirus vector particles are administered.

33. The method of claim 1, wherein the said vector is administered in a volume of about 10 ml or less.

34. The method of claim 1, wherein between 1 and 100 PFU of vector is administered per cell.

35. The method of claim 34, wherein between 10 and 50 PFU of vector is administered per cell.

36. The method of claim 11, wherein said administration is performed at least twice.

37. The method of claim 36, wherein the PFU of the vector in the second administration is different from that in the first administration.

38. The method of claim 36, wherein the second administration follows the first by about six months.

39. The method of claim 36, wherein the second administration follows the first by about one year.

40. The method of claim 1, further comprising the step of tumor resection.

41. The method of claim 40, wherein said tumor resection occurs prior to said administration.

42. The method of claim 41, wherein said administration comprises injection of the residual tumor site.

43. The method of claim 40, wherein said tumor resection is via bronchoscopy.

44. The method of claim 1, further comprising testing said patient for the presence of antibodies reactive with adenovirus.

45. The method of claim 1, further comprising assessing tumor mass via endoscope.

46. The method of claim 45, wherein said endoscope is a bronchoscope.

47. The method of claim 1, wherein said tumor mass is photographed prior to administration.

48. The method of claim 1, further comprising monitoring vector-based toxicity in said patient following administration.

49. A method of inducing cell death in human tumor cells comprising administering directly to a tumor comprised of cells which lack functional p53, an adenovirus vector which does not express functional E1B, wherein the vector further comprises and expresses a DNA sequence encoding wild-type p53 operably linked to the CMV IE promoter, and wherein sufficient wild-type p53 is expressed in the tumor cells to induce cell death resulting in regression of the tumor.

50. The method of claim 49, wherein said tumor cells is a human breast cancer cell.

51. The method of claim 49, wherein said tumor cell is an epithelial cancer cell.

52. The method of claim 49, wherein said tumor cell is a human lung cancer cell.

53. The method of claim 52, wherein said lung cancer cell is a small cell lung carcinoma cell.

54. The method of claim 52, wherein said lung cancer cell is a non-small cell lung carcinoma cell.

55. The method of claim 54, wherein said non-small cell lung carcinoma is an adenocarcinoma cell.

56. The method of claim 54, wherein said non-small cell lung carcinoma cell is a large-cell undifferentiated cell.

57. The method of claim 54, wherein said non-small cell lung carcinoma cell is a squamous carcinoma cell.

58. The method of claim 49, wherein the vector comprises adenovirus type-5 sequences.

59. The method of claim 47, wherein the vector further comprises a polyadenylation signal.

60. The method of claim 59, wherein said the polyadenylation signal is from SV40.

61. The method of claim 49, wherein the vector is a replication-defective adenovirus.

62. The method of claim 61, wherein said vector lacks the E1B coding region and the DNA sequence encoding p53 is substituted for therefore.

63. The method of claim 62, wherein said vector lacks map units 1.3–9.2 of adenovirus type-5, and wherein said DNA sequence is substituted therefor.

64. The method of claim 63, wherein said vector is packaged in an E1-expressing cell line.

65. The method of claim 64, wherein said E1-expressing cell line is the 293 cell line.

66. The method of claim 49, wherein said vector further comprises an origin of replication.

67. The method of claim 66, wherein said origin of replication is viral and is selected from the group consisting of SV40, polyoma, adenovirus, VSV and BPV.

68. The method of claim 67, wherein said origin of replication is from an adenovirus.

69. The method of claim 49, wherein said vector further comprises an enhancer.

70. The method of claim 49, wherein said administration is by direct injection of the tumor.

71. The method of claim 49, wherein said administration is by direct intravenous injection of the tumor.

72. The method of claim 49, wherein said adenovirus composition is administered via intratracheal injection.

73. The method of claim 49, wherein said administration is by infusion over a period of time.

74. The method of claim 73, wherein said period of time is 48 hours.

75. The method of claim 49, wherein about 10^3 to 5×10^{12} adenovirus vector particles are administered.

76. The method of claim 75, wherein about 10^{10} to 5×10^{12} adenovirus vector particles are administered.

77. The method of claim 76, wherein about 10^{10} adenovirus vector particles are administered.

78. The method of claim 76, wherein about 5×10^{12} adenovirus vector particles are administered.

79. The method of claim 49, wherein the said vector is administered in a volume of about 10 ml or less.

80. The method of claim 49, wherein between 1 and 100 PFU of vector is administered per cell.

81. The method of claim 80, wherein between 10 and 50 PFU of vector is administered per cell.

82. The method of claim 49, wherein said administration is performed at least twice.

83. The method of claim 82, wherein the PFU of the vector in the second administration is different from that in the first administration.

84. The method of claim 82, wherein said second administration follows the first by about six months.

85. The method of claim 82, wherein said second administration follows the first by about one year.

86. The method of claim 49, further comprising the step of tumor resection.

87. The method of claim 86, wherein said tumor resection occurs prior to said administration.

88. The method of claim 87, wherein said administration comprises injection of the residual tumor site.

89. The method of claim 86, wherein said tumor resection is via bronchoscopy.

90. The method of claim 49, further comprising testing said patient for the presence of antibodies reactive with adenovirus.

91. The method of claim 49, further comprising assessing tumor mass via endoscope.

92. The method of claim 91, wherein said endoscope is a bronchoscope.

93. The method of claim 49, wherein said tumor mass is photographed prior to administration.

94. The method of claim 49, further comprising monitoring vector-based toxicity in said patient following administration.

* * * * *

22. A recombinant adenovirus which carries an adenovirus vector construct comprising an expression region encoding p53 under the control of a cytomegalovirus IE promoter.

23. The recombinant adenovirus of claim 22, wherein said vector construct further comprises a polyadenylation signal.

24. The recombinant adenovirus of claim 22, wherein said recombinant adenovirus is replication deficient.

25. The recombinant adenovirus of claim 24, wherein said vector construct lacks the E1A and E1B regions.

27. The recombinant adenovirus of claim 24, wherein said expression region replaces said E1A and E1B regions of said vector construct.

28. The recombinant adenovirus of claim 27, wherein said adenovirus has the genome structure of FIG. 1.

36. A recombinant host cell infected with a recombinant adenovirus which carries an adenovirus vector construct comprising an expression region encoding p53 under the control of a cytomegalovirus IE promoter.

37. An adenovirus vector construct comprising an expression region encoding p53 under the control of a cytomegalovirus IE promoter.

38. The adenovirus vector construct of claim 37, further comprising a polyadenylation signal.

39. The adenovirus vector construct of claim 37, wherein said vector construct is replication deficient.

40. The adenovirus vector construct of claim 39, wherein said vector construct lacks the E1A and E1B regions.

41. The adenovirus vector construct of claim 40, wherein said expression region replaces said E1A and E1B regions of said vector construct.

42. The adenovirus vector construct of claim 41, wherein said vector construct has the genome structure of FIG. 1.

9. A method of treating a human cancer patient having a human malignancy comprising administering to said patient an amount of an adenovirus composition effective to inhibit said malignancy, wherein said adenovirus composition comprises an adenovirus vector construct comprising a wild-type p53 gene under the control of a promoter, dispersed in a pharmacologically acceptable solution.

10. The method of claim 9, wherein said patient carries a tumor comprised of malignant cells containing a mutation in their endogenous p53 gene.

11. The method of claim 28, wherein said malignancy is human lung cancer.

12. The method of claim 10, wherein said malignancy is human breast cancer.

13. The method of claim 9, wherein the adenovirus composition comprises replication-defective adenovirus.

24. The method of claim 25, wherein said vector construct lacks adenovirus E1B-coding regions, wherein said p53 gene is substituted therefor.

25. The method of claim 9, wherein the vector construct comprises Adenovirus type 5 sequences.

26. The method of claim 27, wherein said adenovirus vector construct is packaged in an E1-expressing cell line.

27. The method of claim 24, wherein the adenovirus vector construct lacks map units 1.3 to 9.2 of Adenovirus type 5, wherein said p53 gene is substituted therefor.

28. The method of claim 24, wherein said adenovirus vector construct further lacks adenovirus E1A-coding regions.

29. The method of claim 10, wherein said malignancy is an epithelial cancer.

30. The method of claim 11, wherein said lung cancer is a non-small cell lung carcinoma.

31. The method of claim 11, wherein said lung cancer is a small cell lung carcinoma.

32. The method of claim 30, wherein said non-small cell lung carcinoma is an adenocarcinoma.

33. The method of claim 30, wherein said non-small cell lung carcinoma is large-cell undifferentiated.

34. The method of claim 30, wherein said non-small cell lung carcinoma is a squamous carcinoma.

35. The method of claim 26, wherein said E1-expressing cell line is the 293 cell line.

36. The method of claim 23, wherein said vector construct further comprises a polyadenylation signal.

37. The method of claim 36, wherein said polyadenylation signal is an SV40 early polyadenylation signal.

38. The method of claim 9, wherein said adenoviral composition is administered to the patient intravenously.

39. The method of claim 9, wherein said adenoviral composition is administered locally to a tumor site.

40. The method of claim 9, wherein said adenoviral composition is administered via intratracheal injection.

41. The method of claim 9, wherein said adenoviral composition is administered via direct injection of a tumor.

42. The method of claim 9, wherein said adenoviral composition is infused over a period of time.

43. The method of claim 42, wherein said period of time is 48 hours.

44. The method of claim 9, further comprising the step of tumor resection.

45. The method of claim 44, wherein said tumor resection occurs prior to said administration.

46. The method of claim 44, wherein said tumor resection is via bronchoscopy.

47. The method of claim 45, wherein said administration comprises injection of the residual tumor site.

48. The method of claim 9, wherein said composition comprises between about 10^3 and about 5×10^{12} adenovirus.

49. The method of claim 48, wherein said composition comprises between about 10^{10} and about 5×10^{12} adenovirus particles.

50. The method of claim 49, wherein said composition comprises about 10^{10} adenovirus particles.

51. The method of claim 50, wherein said composition comprises about 5×10^{12} adenovirus particles.

52. The method of claim 9, wherein said composition is delivered in a volume of about 10 ml or less.

53. The method of claim 28, wherein said vector construct also lacks the E2 region.

54. The method of claim 28, wherein said vector construct also lacks the E3 region.

55. The method of claim 28, wherein said vector construct also lacks the E4 region.

56. The method of claim 9, wherein said vector construct further comprises an origin of replication.

57. The method of claim 56, wherein said origin of replication is viral and selected from the group consisting of SV40, polyoma, adenovirus, VSV and BPV.

58. The method of claim 57, wherein said origin of replication is from adenovirus.

59. The method of claim 9, wherein the p53 gene is positioned under the control of a CMV, SV40, polyoma, actin, or Adenovirus 2 promoter.

60. The method of claim 10, wherein said amount comprises between 1 and about 100 PFU per cell.

61. The method of claim 60, wherein said amount comprises between about 10 and about 50 PFU per cell.

62. The method of claim 10, wherein the expression of wild-type p53 is approximately 14-times higher than the expression of resident p53 in said cell.

63. The method of claim 9, wherein said vector construct comprises an enhancer.

64. The method of claim 9, further comprising testing said patient for the presence of antibodies reactive with adenovirus.

65. The method of claim 10, further comprising assessing the tumor via endoscope.

66. The method of claim 65, wherein said endoscope is a bronchoscope.

67. The method of claim 10, wherein said tumor is photographed prior to administration.

68. The method of claim 9, wherein said administration is performed at least twice.

69. The method of claim 68, wherein the PFU of the adenovirus composition in the second administration differs from that of the first administration.

70. The method of claim 9, further comprising monitoring vector-based toxicity in said patient following administration.

71. The method of claim 68, wherein the second administration follows the first by about six months.

72. The method of claim 68, wherein the second administration follows the first by about one year.

73. The method of claim 59, wherein the promoter is a CMV promoter.

74. A method of treating a human patient having cancer comprising administering to the patient a pharmacologically acceptable composition including an adenovirus vector having a DNA segment encoding a wild-type p53 polypeptide under the control of a CMV promoter, wherein the composition inhibits the patient's cancer.

75. A pharmaceutical composition comprising:

(a) a recombinant adenovirus containing an adenovirus vector construct comprising an expression region encoding p53 under the control of a promoter; and

(b) a pharmaceutically acceptable carrier, excipient, or diluent.

76. The pharmaceutical composition of claim 75, wherein the promoter is a cytomegalovirus IE promoter.

77. The pharmaceutical composition of claim 75, wherein said vector construct further comprises a polyadenylation signal.

78. The pharmaceutical composition of claim 77, wherein said recombinant adenovirus is replication deficient.

79. The pharmaceutical composition of claim 78, wherein said vector construct lacks the E1A and E1B regions.

80. The pharmaceutical composition of claim 79, wherein said expression region replaces said E1A and E1B regions of said vector construct.

81. The pharmaceutical composition of claim 80, wherein said adenovirus has the genome structure of FIG. 1.

82. A pharmaceutical composition comprising:

(a) a recombinant adenovirus containing an adenovirus vector construct comprising an expression region encoding p53 under the control of a cytomegalovirus IE promoter; and

(b) a pharmaceutically acceptable carrier, excipient or diluent.

83. The pharmaceutical composition of claim 82, wherein said vector construct further comprises a polyadenylation signal.

84. The pharmaceutical composition of claim 83, wherein said recombinant adenovirus is replication deficient.

85. The pharmaceutical composition of claim 84, wherein said vector construct lacks the E1A and E1B regions.

86. The pharmaceutical composition of claim 85, wherein said expression region replaces said E1A and E1B regions of said vector construct.

87. The pharmaceutical composition of claim 86, wherein said adenovirus has the genome structure of FIG. 1.

88. A pharmaceutical composition comprising:

(a) a recombinant adenovirus containing an adenovirus vector construct comprising an expression region encoding p53 under the control of a promoter, wherein the promoter provides expression sufficient to inhibit tumor cell growth *in vivo*; and

(b) a pharmaceutically acceptable carrier, excipient, or diluent.

89. The pharmaceutical composition of claim 88, wherein the promoter is a cytomegalovirus promoter.

90. The pharmaceutical composition of claim 88, wherein said vector construct further comprises a polyadenylation signal.

91. The pharmaceutical composition of claim 90, wherein said recombinant adenovirus is replication deficient.

92. The pharmaceutical composition of claim 91, wherein said vector construct lacks the E1A and E1B regions.

93. The pharmaceutical composition of claim 92, wherein said expression region replaces said E1A and E1B regions of said vector construct.

94. The pharmaceutical composition of claim 93, wherein said adenovirus has the genome structure of FIG. 1.

95. A pharmaceutical composition for systemic administration comprising:

(a) a recombinant adenovirus containing an adenovirus vector construct comprising an expression region encoding p53 under the control of a promoter; and

(b) a carrier, excipient, or diluent, that is pharmaceutically acceptable for systemic administration.

96. The pharmaceutical composition of claim 95, wherein the promoter is a cytomegalovirus promoter.

97. The pharmaceutical composition of claim 95, wherein said vector construct further comprises a polyadenylation signal.

98. The pharmaceutical composition of claim 97, wherein said recombinant adenovirus is replication deficient.

99. The pharmaceutical composition of claim 98, wherein said vector construct lacks the E1A and E1B regions.

100. The pharmaceutical composition of claim 99, wherein said expression region replaces said E1A and E1B regions of said vector construct.

101. The pharmaceutical composition of claim 100, wherein said adenovirus has the genome structure of FIG. 1.

22. A method of treating a human cancer patient having a human malignancy, comprising administering regionally to said patient an amount of an adenovirus composition effective to prevent growth of malignant cells, wherein said adenovirus composition comprises an adenovirus vector construct comprising a p53 gene, dispersed in a pharmacologically acceptable solution.

23. The method of claim 22, wherein said adenoviral composition is administered to the patient by infusion over a period of time.

24. The method of claim 23, wherein said period of time is about 48 hours.

25. The method of claim 22, wherein said amount comprises between about 10^3 to about 5 x 10^{12} adenovirus particles.

26. The method of claim 25, wherein said amount comprises between about 10^3 to about 10^6 adenovirus particles.

27. The method of claim 25, wherein said amount comprises between about 1 x 10^{10} to about 5 x 10^{12} adenovirus particles.

28. The method of claim 25, wherein said amount comprises about 1 x 10^{10} virus particles.

29. The method of claim 25, wherein said amount comprises about 3 x 10^{10} virus particles.

30. The method of claim 25, wherein said amount comprises about 5 x 10^{12} adenovirus particles.

31. The method of claim 22, further comprising at least a second administration of the adenoviral composition.

32. The method of claim 31, further comprising at least a third administration of the adenoviral composition.

33. The method of claim 32, wherein the third administration occurs at least about one day after the second administration.

34. The method of claim 32, wherein the third administration occurs about one day after the second administration.

35. The method of claim 32, wherein said first, second, and third administrations are each given on three consecutive days.

36. The method of claim 22, further comprising resecting a tumor of said cancer patient.

37. The method of claim 22, wherein said resecting occurs prior to said administering.

38. The method of claim 22, wherein said adenoviral composition further comprises phosphate-buffered saline with about 1% (v/v) glycerol.

39. The method of claim 22, wherein said adenoviral composition is delivered in a volume of about 10 ml or less.

40. The method of claim 22, wherein the p53 gene is under the control of a CMV promoter.

41. The method of claim 22, wherein said growth is prevented by apoptosis.

42. A method of treating a human cancer patient comprising administering intravenously to said patient, in at least three administrations, an amount of an adenovirus composition effective to prevent growth of malignant cells, wherein said adenovirus composition comprises an

adenovirus vector construct comprising a p53 gene, dispersed in a pharmacologically acceptable solution.

43. The method of claim 42, wherein said adenoviral composition is administered to the patient by intravenous infusion over a period of time.

44. The method of claim 43, wherein said period of time is about 48 hours.

45. The method of claim 42, wherein said amount comprises between about 10^3 to about 5 x 10^{12} adenovirus particles.

46. The method of claim 45, wherein said amount comprises between about 10^3 to about 10^6 adenovirus particles.

47. The method of claim 45, wherein said amount comprises between about 1 x 10^{10} to about 5 x 10^{12} adenovirus particles.

50. The method of claim 42, wherein the third administration occurs at least about one day after the second administration.

51. The method of claim 42, wherein the third administration occurs about one day after the second administration.

52. The method of claim 42, wherein said first, second, and third administrations are each given on three consecutive days.

53. The method of claim 42, further comprising resecting a tumor of said cancer patient.

54. The method of claim 42, wherein said resecting occurs prior to said administering.

55. The method of claim 42, wherein said adenoviral composition further comprises phosphate-buffered saline with about 1% (v/v) glycerol.

56. The method of claim 42, wherein said adenoviral composition is delivered in a volume of about 10 ml or less.

57. The method of claim 42, wherein the p53 gene is under the control of a CMV promoter.

58. The method of claim 42, wherein said growth is prevented by apoptosis.

59. A method of treating a human cancer patient comprising instilling intratracheally to said patient, in at least three administrations, an amount of an adenovirus composition effective to effective to prevent growth of malignant cells, wherein said adenovirus composition comprises an adenovirus vector construct comprising a p53 gene, dispersed in a pharmacologically acceptable solution.

60. The method of claim 59, wherein said adenoviral composition is administered to the patient by infusion over a period of time.

61. The method of claim 60, wherein said period of time is about 48 hours.

62. The method of claim 59, wherein said amount comprises between about 10^3 to about 5 x 10^{12} adenovirus particles.

63. The method of claim 59, wherein said amount comprises between about 10^3 to about 10^6 adenovirus particles.

64. The method of claim 63, wherein said amount comprises between about 1 x 10^{10} to about 5 x 10^{12} adenovirus particles.

65. The method of claim 63, wherein said amount comprises about 1 x 10^{10} virus particles.

66. The method of claim 63, wherein said amount comprises about 3 x 10^{10} virus particles.

67. The method of claim 63, wherein said amount comprises about 5 x 10^{12} adenovirus particles.

70. The method of claim 59, wherein the third administration occurs at least about one day after the second administration.

71. The method of claim 59, wherein the third administration occurs about one day after the second administration.

72. The method of claim 59, wherein said first, second, and third administrations are each given on three consecutive days.

73. The method of claim 59, further comprising resecting a tumor of said cancer patient.

74. The method of claim 59, wherein said resecting occurs prior to said administering.

75. The method of claim 59, wherein said adenoviral composition further comprises phosphate-buffered saline with about 1% (v/v) glycerol.

76. The method of claim 59, wherein said adenoviral composition is delivered in a volume of about 10 ml or less.

77. The method of claim 59, wherein the p53 gene is under the control of a CMV promoter.

78. The method of claim 59, wherein said growth is prevented by apoptosis.

79. A method of treating a human cancer patient having a tumor, comprising administering by direct injection of the tumor, in at least three administrations, an amount of an adenovirus composition effective to inhibit growth of tumor cells, wherein said adenovirus composition comprises an adenovirus vector construct comprising a p53 gene, dispersed in a pharmacologically acceptable solution.

80. The method of claim 79, wherein said amount comprises between about 10^3 to about 5 x 10^{12} adenovirus particles.

81. The method of claim 80, wherein said amount comprises between about 10^3 to about 10^6 adenovirus particles.

82. The method of claim 80, wherein said amount comprises between about 1 x 10^{10} to about 5 x 10^{12} adenovirus particles.

83. The method of claim 82, wherein said amount comprises about 1 x 10^{10} adenovirus particles.

84. The method of claim 82, wherein said amount comprises about 3 x 10^{10} adenovirus particles.

85. The method of claim 82, wherein said amount comprises about 5 x 10^{12} adenovirus particles.

88. The method of claim 79, wherein the third administration occurs at least about one day after the second administration.

89. The method of claim 79, wherein the third administration occurs about one day after the second administration.

90. The method of claim 79, wherein said first, second, and third administrations are each given on three consecutive days.

91. The method of claim 79, further comprising resecting at least part of the tumor of said cancer patient.

92. The method of claim 79, wherein said resecting occurs prior to said administering.

93. The method of claim 79, wherein said adenoviral composition is dispersed in phosphate-buffered saline with about 1% (v/v) glycerol.

94. The method of claim 79, wherein said adenoviral composition is administered in a volume of about 10 ml or less.

95. The method of claim 79, wherein the p53 gene is under the control of a CMV promoter.

96. The method of claim 79, wherein said tumor cells are killed by apoptosis.

97. The method of claim 91, further comprising administering the adenoviral composition to the residual tumor site via direct injection.

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Introgen Therapeutics, Inc. (ticker: ingn, exchange: NASDAQ) News Release - 2/21/02

Introgen Begins Branding INGN 201 With Award of ADVEXIN (R) Registered Trademark

AUSTIN, Texas, Feb 21, 2002 /PRNewswire-FirstCall via COMTEX/ -- Introgen Therapeutics, Inc. (Nasdaq: INGN) announced today that it has received notification from the U.S. Patent and Trademark Office that the name ADVEXIN (R) is now a registered trademark in connection with Introgen's novel gene based drug for cancer. Introgen notified the Federal Food and Drug Administration (FDA) of the mark. The mark is registered in the United States, the European Community, and numerous other countries. The gene therapeutic ADVEXIN(R) is currently the subject of two randomized and controlled Phase 3 clinical trials for the treatment of head and neck cancer as well as numerous other clinical studies for a variety of solid tumors such as lung, brain, prostate, breast and bladder cancers.

Max W. Talbott, Ph.D., Introgen's senior vice president of worldwide commercial development said, "After almost twenty clinical studies, the product development phase of Adenoviral p53 ADVEXIN(R) is nearing completion. Introgen is now implementing pre-marketing strategies in preparation for the approval of this revolutionary cancer therapeutic."

Introgen is a leader in the development and production of gene-based drugs for the treatment of cancer and other diseases. Introgen's product candidates engage precise molecular targets to produce a highly specific therapeutic effect. By selectively killing cancer cells and harnessing natural protection mechanisms, Introgen's product candidates may be less toxic than conventional treatments. Introgen specializes in combining appropriate gene delivery systems and

therapeutic genes to make its gene-based drugs. Introgen is currently conducting two controlled, randomized Phase 3 clinical trials with its lead product, INGN 201, ADVEXIN(R), for the treatment of head and neck cancer. Introgen's gene therapeutics have been used in approximately twenty clinical trials worldwide either alone or in combination with conventional treatments such as chemotherapy, surgery and radiotherapy. Introgen is also conducting a Phase 2 clinical trial for INGN 201, ADVEXIN(R), in lung cancer and Phase 1 trials for INGN 201, ADVEXIN(R), in additional cancer indications including prostate, ovarian, bladder, brain, and breast cancer. New applications using the human immune system with INGN 201, ADVEXIN(R), are being explored. Introgen's second product candidate, INGN 241 (Adenoviral-mda7) for the treatment of solid tumors, is in Phase 1 clinical development.

Certain statements in this press release that are not strictly historical may be "forward-looking" statements, which involve risks and uncertainties. Such forward-looking statements include, but are not limited to, those relating to the success of Introgen's clinical development program. Introgen's actual results may differ from those described in this press release due to risks and uncertainties that exist in Introgen's operations and business environment, including, without limitation, Introgen's stage of product development and its limited experience in the development of gene-based drugs in general, uncertainties related to clinical trials, Introgen's dependence upon proprietary technology and patent protection, Introgen's history of operating losses and accumulated deficits, Introgen's reliance on collaborative relationships, Introgen's ability to obtain appropriate regulatory approvals, and market acceptance of Introgen's product candidates, as well as other risks detailed in Introgen's filings with the Securities and Exchange Commission, including its annual report of Form 10-K filed with the Securities and Exchange Commission on September 19, 2001 and its quarterly report of Form 10-Q filed with the Securities and Exchange Commission on November 14, 2001. Introgen undertakes no obligation to publicly release the results of any revisions to any forward-looking statements that reflect events or circumstances arising from the date hereof.

Editor's Note: For more information on Introgen Therapeutics, or for a menu of archived press releases, please visit Introgen's Website at: www.introgen.com or call Introgen's toll-free Investor Relations hotline at 1-877-776-GENE (4363).

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Introgen Therapeutics, Inc. (ticker: ingn, exchange: NASDAQ) News Release - 7/31/02

Key Patent Obtained for Introgen's Phase 3 Product; Introgen Is Exclusive Licensee of Patent for ADVEXIN(R) Gene Therapeutic

AUSTIN, Texas, Jul 31, 2002 /PRNewswire-FirstCall via COMTEX/ -- Introgen Therapeutics, Inc. (Nasdaq: INGN) and The University of Texas M. D. Anderson Cancer Center announced today that the United States Patent and Trademark Office has issued to The Board of Regents of The University of Texas System United States patent number 6,410,010. The patent broadly covers all adenoviral p53 compositions, therapeutic and otherwise, that express adequate p53 in amounts sufficient to suppress the growth of or kill cancer cells in patients including ADVEXIN, Introgen's p53 cancer therapeutic. The patent also covers adenoviral p53 that incorporates a specific type of promoter that helps cells to express the p53 tumor suppressor gene, the key gene in the ADVEXIN therapeutic. Introgen Therapeutics is the exclusive licensee of this patent and is currently enrolling head and neck cancer patients in two phase 3 trials evaluating ADVEXIN gene therapy.

David L. Parker, Ph.D., J.D., Introgen's vice president of intellectual property said, "We have previously received patents on the clinical use and the manufacture of the product candidate. Now we have patented the ADVEXIN gene therapeutic directly as a composition of matter. This is therefore a key patent in our ever-expanding portfolio of intellectual property, one that further demonstrates our leadership in the gene therapy arena."

ADVEXIN is covered by 8 issued U.S. patents in virtually all relevant areas including compositions, methods of administering the product in virtually any form alone and with the most widely used treatments, and process and production.

ADVEXIN, formerly designated by Introgen as INGN 201, is a patented cancer therapeutic incorporating the p53 tumor suppressor gene in an adenoviral delivery system. ADVEXIN is designed to use the p53 gene to kill cancer cells and to stop tumor growth, without harming normal cells, in cancer patients with both normal and damaged p53 genes. The p53 gene interferes with cancer cells because it is a tumor suppressor gene and carries instructions to make a protein that reacts with the damaged DNA of a cancer cell. Specifically, the p53 protein activates one of two pathways in these cells, to either stop growth by arresting cancer cell growth or inducing cancer cell death via a process of programmed cell death, called apoptosis. Both processes provide an important brake to the development of certain cancers.

Introgen controls a number of U.S. patents that relate to adenoviral p53 as well as p53 combination therapy. Three patents cover gene therapy of cancer using a p53 tumor suppressor gene in combination with one or more chemotherapeutic drugs, radiation therapies, or other agents that have a damaging effect on the DNA of cancer cells. Two of these patents cover the clinical use of the p53 gene before, during or after treatment with chemotherapy or radiotherapy, and the third covers therapy using the p53 gene in combination with a class of chemotherapy agents called DNA repair inhibitors. An additional patent covers the treatment of cancer with adenoviral p53 to kill tumor cells and promote tumor regression.

Introgen is a leader in the development and production of gene-based drugs for the treatment of cancer and other diseases. Introgen maintains integrated research, development, manufacturing, clinical and regulatory departments and operates a commercial-scale, cGMP manufacturing facility. Through more than 20 clinical trials, the company has treated hundreds of patients with thousands of doses of its lead product candidate, ADVEXIN(R) adenoviral p53 therapy. Introgen is conducting two controlled, randomized phase 3 clinical trials with ADVEXIN for the treatment of head and neck cancer, a phase 2 study in breast cancer, as well as phase 1 trials in prostate, ovarian, bladder, and brain cancers, and completed phase 2 studies in lung and head and neck cancers.

Certain statements in this press release that are not strictly historical may be "forward-looking" statements, which involve risks and uncertainties. Such forward-looking statements include, but are not limited to, those relating to the success of Introgen's clinical development program, including ADVEXIN or the patent protection of ADVEXIN. There can be no assurance that Introgen will be able to commercially develop gene-based drugs, that necessary regulatory approvals will be obtained or that any clinical trials or studies undertaken will be successful or that the proposed treatments will prove to be safe and/or effective. The actual results may differ from those described in this press release due to risks and uncertainties that exist in Introgen's operations and business environment, including, but without limitation, Introgen's stage of product development and the limited experience in the development of gene-based drugs in general, its dependence upon proprietary technology and current competition, history of operating losses and accumulated deficits, Introgen's reliance on collaborative relationships, and uncertainties related to clinical trials, safety, efficacy, the ability to obtain the appropriate regulatory approvals, patent protection and market acceptance, as well as other risks detailed from time to time in Introgen's filings with the Securities and Exchange Commission, including its quarterly report on Form 10-Q filed on May 15, 2002. Introgen undertakes no obligation to publicly release the results of any revisions to any forward-looking statements that reflect events or circumstances arising from the date hereof.

Editor's Note: For more information on Introgen Therapeutics, or for a menu of archived news releases, please visit Introgen's Website at www.introgen.com .

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